THE USE OF STABLE ISOTOPE RATIOS OF CARBON AND NITROGEN TO ELUCIDATE PELAGIC MARINE FOODWEBS OF THE BENGUELA AND AGULHAS BANK REGIONS OF SOUTH AFRICA

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ABSTRACT

Isotope assessments of foodweb relationships amongst pelagic organisms may be influenced by their lipid content, since lipids are more depleted in ^{13}C than other biochemical compounds. This is particularly important for plankton which show a greater decrease in $\delta^{13}C$ caused by the failure to remove lipids during sample preparation, than the muscle tissue of pelagic fish species. Lipid removal is important for those fish species whose lipid content and magnitude of diet-consumer fractionation are simultaneously related to their size.

The period required for pelagic fish to isotopically reflect a new diet is slow, of the order of months and years, and may depend on the diet and the magnitude of isotopic change displayed. It is likely that this rate decreases as the fish approach isotopic equilibrium with the new food source.

Measurements of the stable isotope ratios of carbon and nitrogen were obtained for different size-fractions of plankton and three species of pelagic fish, Engraulis capensis Gilchrist Etrumeus whiteheadi Wongratana and Sardinops ocellatus (Pappe), from the Benguela Current system off the west coast of South Africa and the Agulhas bank region off the south coast. Plankton tend to have more positive $\delta^{13}C$ and $\delta^{15}N$ values with increasing size. The data are consistent with the hypothesis that larger plankton feed further up the food web than smaller plankton. There are two isotopically distinct foodwebs from phytoplankton to fish from the southern Benguela ecosystem, one more enriched in ^{13}C than the other. The less ^{13}C enriched foodweb is isotopically similar to that encountered amongst the organisms from the Agulhas Bank. The $\delta^{13}C$ and $\delta^{15}N$ values of fish muscle and bone collagen tissues tend to be more positive than those for plankton. The general isotopic similarity amongst the three species of fish suggests that they occupy similar positions within the foodweb. E. whiteheadi and S. ocellatus appear to have consumed more phytoplankton than E. capensis. The isotope values for the fish tissues become more negative with increasing fish length for E. capensis and E. whiteheadi, in contrast to the opposite tendency with increasing plankton size and to the fish tissues. The $\delta^{13}C$ content of the muscle tissue of S. ocellatus becomes more positive with increasing fish length. The relationships with fish length may be the result of dietary switching, or alternatively may have a physiological basis (resulting from the different metabolic activities of different sized fish). The fractionation of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios caused by the formation of muscle tissue is different from that for bone collagen. Bone collagen is richer in ^{13}C than muscle tissue whereas muscle is richer than bone collagen in ^{15}N. The tissue with more negative $\delta^{13}C$ or $\delta^{15}N$ values shows the stronger correlation with fish length (negative for E. capensis and E. whiteheadi, positive for S. ocellatus).

CONTENTS

CHAPTER 1

INTRODUCTION

Adequate management of the commercial fishery requires a knowledge of the diets of commercially important fish species, since food is an important factor regulating their growth, abundance and migration. Intermediate microphagous clupeids display a high degree of opportunism; they are capable of alternating their feeding strategies to efficiently utilise the available trophic spectrum. The diets of these fish depend upon their size and the size, concentration and availability of their prey (Koslow 1981; Angelescu 1982; James 1987; James & Findlay 1989). Within the pelagic foodweb, larger organisms are able to consume a greater size range of organisms smaller than themselves (Koslow 1981; Angelescu 1982). Thus pelagic food-webs may be modelled in terms of organism-size relationships rather than trophic interactions (Cousins 1980, 1985; Azam et al. 1983; Platt 1985; Moloney & Field 1989), since it is difficult to assign organisms of different ages or species from different habitats, to the same trophic level.

Disparities existing between data sets may result from subjective methods employed to estimate prey abundance in gut content samples (e.g. numerical counts). Furthermore gut content analyses are themselves subject to temporal bias associated with the diurnal migrations of these fishes (which may differ with fish age and reproductive state (Shelton & Hutchings 1982; Hampton et al. 1985; James 1987), area of capture (De Mendiola 1971), season (King & Macleod 1976) and spawning activity (James 1988), or combinations of these factors. Less subjective, less laborious methods are required to estimate the integrated average diets of important fish species.

Elements may exist in both stable and unstable (radioactive) forms. Two elements with the same atomic number and different mass numbers are said to be isotopes of one another (Masterton & Sowinski 1977). Most elements of biological interest have two or more stable isotopes, one usually in much greater abundance than the other (Ehleringer & Rundel 1989). The stable isotopes of carbon, ^{12}C and ^{13}C, exist in nature in the proportions; 98.89 : 1.1 % by atoms. Nitrogen exists as two stable isotopes, ^{14}N (99.63 %) and ^{15}N (0.37% by atoms). The behaviour of isotopes of an element in most chemical and

biochemical reactions are unaffected, save for slight effects due to differences in mass. Isotope ratios change as elements cycle through the biosphere. In this study, we are interested in how variability in the ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in marine pelagic organisms can be used as a tool to trace C and N flow in pelagic ecosystems. Measurements of these isotope ratios are used to examine how they reflect the diets of three small pelagic school fish species from the southern Benguela ecosystem (Cape anchovy, Engraulis capensis Gilchrist, redeye roundherring, Etrumeus whiteheadi Wongratana and South African pilchard, or sardine (to which they are referred throughout this thesis), Sardinops ocellatus (Pappe), as well as the diets of their plankton prey. Furthermore, an attempt is made to clarify the isotopic foodweb by relating the data to organism size. Previous isotopic studies of pelagic foodwebs have concentrated on differences existing at discrete trophic levels.

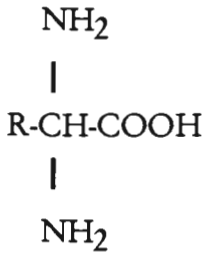
1.1 PROTEIN CHEMISTRY

Proteins are nitrogenous organic substances produced by and associated with living matter (Conn & Stumpf 1976). The approximate composition of proteins may be expressed as:

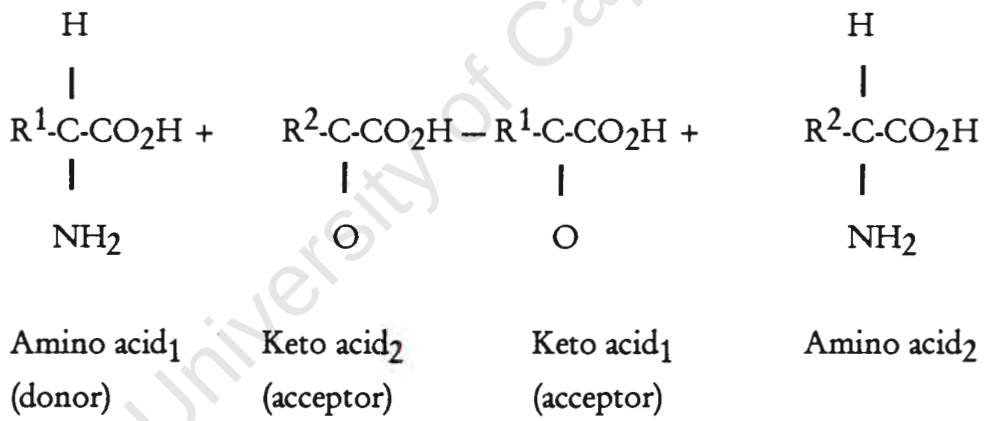
Elements always present:	Elements not always present:
Carbon = 50%	Sulphur = 0.3%
Hydrogen = 7%	Phosphorous = 0.3%
Nitrogen = 16%	
Oxygen = 25%	

Thus the C/N ratio of protein is about 3:1.

Proteins are synthesised in nature from amino acids i.e. compounds containing both an amino (NH_2) and carboxylic acid (COOH) function (Conn & Stumpf 1976). Nearly all the amino acids in proteins are α-amino acids, where the amino group is present on the α-carbon atom (Plummer 1978). These groups are attached to a carbon skeleton in the following arrangement:



The carboxyl group of one amino acid combines with the amino group of another to form a long chain of amino acids in a sequence designated by mRNA in the cell (Conn & Stumpf 1976). The synthesis of amino acids in plants and animals requires the provision of a nitrogen-containing source and a carbon skeleton. The biosynthesis of most amino acids is accomplished by a process of transamination (Conn & Stumpf 1976). Transamination involves both the breakdown and synthesis of many amino acids. An amino group from one amino acid (the donor) is transferred to a keto acid (the carbon skeleton of another amino acid). The original amino donor becomes a keto acceptor:



The transamination reaction involving the deamination of glutamate by glutamate dehydrogenase, and resulting in the formation of glutamic acid, is particularly important, since glutamic acid is specifically permeable to the inner mitochondrial membrane. The liver enzyme, glutamic dehydrogenase, is present in the mitochondria, and catalyzes the deamination of L-glutamate to α-ketoglutaric acid, NH_3 and NADH. The α-ketoglutaric acid thus formed becomes a keto-acceptor for other donor amino acids (Conn & Stumpf 1976). Furthermore, glutamic acid itself may serve as a precursor of other amino acids, such as proline and ornithine.

Some amino acids can be synthesised by the animal (if in short supply in their dietary provision) and these are known as non-essential amino acids. Essential amino acids are those that must be supplied by the diet.

Transamination allows for the formation of the amino acids required by an organism to function. Carbon skeletons formed by the degradation of protein tissue can be reutilised during tissue turnover.

In plants and microorganisms, the nitrate ion (NO_3^-) and sometimes ammonia (NH_3) are used for the biosynthesis of amino acids, proteins and nucleic acids. In the higher animals, amino acids arise mainly from the digestion of dietary proteins (Conn & Stumpf 1976; Plummer 1978).

1.1.1 Muscle tissue

Muscle constitutes the largest mass of protein in the body (Waterlow et al. 1978). The myofibrillar proteins make up about 70% of muscle intracellular protein (or 25% of body protein). The myofibrils are made up of contractile proteins (myosin and actin) and others with a more regulatory function (tropomyosin and troponin) and those with a structural function (α-actinin and M-protein) (Waterlow et al. 1978). These six proteins account for over 95% of myofibrillar protein. Myosin alone makes up about 60% of myofibrillar mass. The thick filaments are mostly made up of myosin, while actin makes up the most part of the thin filaments (Schmidt-Nielson 1979).

Myosin is composed of two large (heavy chain) subunits and four smaller ones (light chain) (Waterlow et al. 1978). Differences between fast (white) skeletal muscle and slow (red) muscles are due to differences in the structure of the heavy chains and the number of light chain types they contain (Waterlow et al. 1978). The activity of ATP (the immediate energy source enabling contraction), is associated with a lower proportion of light chain.

The amino acids, lysine, threonine and histidine have the highest concentrations relative to the other essential amino acids in rat muscle (Waterlow et al. 1978). Lysine and

threonine are said not to undergo transamination (Waterlow 1978), but may donate nitrogen to the pool (Hare 1988).

1.1.2 Bone collagen tissue

Bone is made up of an organic component (collagen) and an inorganic component (apatite). Collagen is the most abundant protein in the body, making up about 20-25% of total body protein in mammals (Waterlow et al. 1978). About half of this collagen occurs in bone and muscle and most of the rest in skin and tendon.

Collagen is a structurally unique fibrillar substance, formed by the covalent cross-linking of triple stranded units (three polypeptide chains) resulting in a particularly stable coiled formation (triple helix) (Waterlow et al. 1978). The main constituent amino acids are glycine (about 33%) and proline (about 25%) which are non-essential amino acids. About half of the proline residues are hydroxylated forming hydroxyproline which is essential for the stability of the triple helix at temperatures in the body. In contrast to muscle tissue, lysine and threonine form only a small percentage of bone collagen protein, but of the essentials, lysine has the greatest concentration (if hydroxylysine is included).

1.2 TISSUE TURNOVER

The isotopic composition of an animal's tissues reflects its integrated diet over time and varies with changes in dietary composition at a rate dependent upon the tissue turnover time (Fry & Arnold 1982; Boutton et al. 1988). The longer the tissue turnover time of an organism, the longer the period of isotopic integration.

Turnover is a general term used to describe the process of renewal or replacement of a given substance. This may involve its production or disappearance (Waterlow et al. 1978, Shipley & Clark 1972). Thus a discussion of protein synthesis or breakdown or both is appropriate to turnover. Protein synthesis joins together all the amino acid residues which remain unchanged within the protein molecule until they are released together by the process of protein degradation (Waterlow et al. 1978). Protein molecules differ in their rate of turnover in an animal (Conn & Stumpf 1976).

Assuming that the process of protein degradation is a random process where the molecules in the pool have an equal chance of being selected for breakdown, turnover time may be expressed as the amount of material (as a fraction of the pool) transferred per unit time. Thus, in terms of kinetics, there are two types of turnover. One is a first order process, or a constant fraction process, in which a constant fraction of the pool is turned over in unit time. The other is a zero order process (constant amount process), in which a constant amount of metabolite is turned over in unit time, regardless of changes in pool size (Waterlow 1978). The distinction between the two aids our understanding of protein turnover in the body as a whole, within individual tissues, or constituent tissue proteins. Protein breakdown is not always a random process and some cells or proteins have a predetermined lifespan (Waterlow et al. 1978)). Nevertheless, the flow of a specific carbon atom through the body is related to the life span of the protein in which it is found.

In humans and other mammals, amino acids are taken up at different rates by different tissues. Amino acids from the food are taken up into the body water, distributed and assimilated first by those metabolically active tissues that turn over rapidly (Waterlow et al. 1978), such as the liver, kidneys and other vital organs (Conn & Stumpf 1976; Tieszen et al. 1983). They are later redistributed (after protein degradation in these tissues and subsequent release of the amino acids to the protein pool) amongst less metabolically active tissues, which have a slower turnover rate (Waterlow et al. 1978), such as muscle tissue and bone collagen (Tieszen et al. 1983). Tieszen et al. (1983) found the half life of the muscle tissue of gerbils to be 27.6 days. Conn & Stumpf (1976) report the half-lives of muscle protein and collagen (presumably in humans) to be about 180 and 1000 days respectively. Amino acids may be reutilised before they are released from the body. Some amino acids are reutilised more so than others, e.g. labelled lysine has been found to have a relatively long half life in plasma albumin (Waterlow et al. 1978). The extent of reutilisation of an amino acid will depend on the turnover rate of the protein and the proportion of that amino acid in that protein.

Within muscle tissue, different myofibrillar proteins have different rates of turnover. Muscles containing red oxidative fibres have a higher intensity of protein synthesis (about twice as fast) than those containing white glycolytic fibres (Waterlow et al. 1978).

The rate of collagen turnover is slower than other proteins, due to its unique stable triple-helical structure (Conn & Stumpf 1976; Waterlow et al. 1978). Unique precursor enzymes, the collagenases, are required to cleave the triple helix and denature the polypeptides to a state where they are susceptible to other protease activity. Degradation of the peptides by proteolysis mostly results in the liberation of free hydroxyproline which is either oxidised or excreted in the urine. Collagen can be divided into two components, a soluble portion (in NaCl solution or dilute acid) and an insoluble portion. The relative solubility of a collagen fibre reflects its age and stability (Waterlow et al. 1978). The younger and more soluble collagen fibres are mostly involved in breakdown. About 2/3rds of soluble collagen reaches the insoluble stage and about 1/3rd is degraded.

Animal size is important since smaller short-lived organisms have faster metabolic rates than larger organisms (Schmidt-Nielsen 1984), and therefore, faster rates of turnover. Thus smaller organisms can be expected to be more isotopically sensitive to short-term changes in diet than larger, longer-lived, organisms. Furthermore, in young rapidly growing animals synthesis and resorption of amino acids is fast, but gross synthesis exceeds net resorption. Accordingly, incorporation of dietary amino acids is significantly more important than reincorporation of amino acids. The rate of whole body synthesis of protein is slower in older individuals. This reduction is due to a decrease in the synthesis rates (measured as the fractional synthesis rate, FSR) of some tissues more than others. The proteins with the slowest breakdown rates show the greatest fall in FSR (Waterlow et al. 1978). The fall in FSR of muscle tissue with age seems to result more from sarcoplasmic proteins than myofibrillar proteins (Waterlow et al. 1978).

There are many reasons or combinations of reasons for the decline in tissue turnover with growth, including the following:

The concentration of ribosomes (i.e. cytoplasmic organelles which form the matrix upon which protein synthesis can take place) declines.

FSR declines in those tissues making up a large percentage of the body.

In muscle tissue, there is a decline in FSR of sarcoplasmic proteins and some decline of that in myofibrillar proteins.

In bone collagen, the proportion of soluble collagen decreases.

Many experiments involved with chemical transfer to and from the body assume that the pool they are dealing with is a single pool of homogeneous atoms. No such pool exists and there are distinguishable amino acid pools in extra- and intracellular compartments (Shipley & Clark 1972). Thus carbon and nitrogen may be viewed as belonging to two different elemental pools. Their steady state may differ, even in the same tissues. Furthermore, their turnover is influenced by the multitude of deamination and transamination processes that occur during the incorporation of amino acids into the body. Nevertheless, single pool kinetics is useful for understanding more complex systems.

1.3 ISOTOPES IN NATURE

In order to understand how stable isotopes reflect the trophic positions and foodweb relationships amongst pelagic organisms, one requires a knowledge of the mechanisms involved, from the base of the foodweb to the higher trophic levels.

1.3.1 Terminology and measurement

Measuring the absolute isotopic composition of C and N (% by atoms) is not as reliable or convenient as measuring isotopic differences between a sample relative to a standard. Most ecological studies express the stable isotope ratios of carbon and nitrogen as a part per thousand ($^{o}/_{oo}$), designated by the Greek letter, δ, according to the equation:

$$\delta X = ((R_{sample} - R_{standard})/R_{standard}) \times 1000 \ (^{o}/_{oo}),$$

where X = ^{13}C or ^{15}N, R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and standard = Peedee Belemnite carbonate (PDB) for $\delta^{13}C$ (Craig 1953), and atmospheric nitrogen (Air) for $\delta^{15}N$ (Hoefs 1980). PDB, a cretaceous marine fossil (Belemnite) from the Peedee formation in South Carolina, is no longer used, but has been replaced by other materials which have been

standardised accordingly (Ehleringer & Rundel 1989). PDB has more ^{13}C than most living things, so $\delta^{13}C$ values for the organisms we measure are negative. Air has less ^{15}N than most organic material, so organisms usually have positive $\delta^{15}N$ values. Nevertheless, an increase in the δ value represents an increase in the relative proportion of the heavier isotopes, ^{13}C or ^{15}N, i.e. isotopic enrichment. When a substance has less ^{13}C or ^{15}N than another related material, we say it is isotopically depleted relative to that material. Isotopic enrichment and depletion are relative terms and are a result of isotopic fractionation. The terminology "more positive" and "more negative" are used with the greatest frequency during this thesis (particularly for consumer species) in order to avoid the suggestion of a chemical relationship between two samples or sets of data.

1.3.2 Fractionation

Fractionation results from isotope effects produced because isotopes with a greater mass number react more slowly than their corresponding counterparts with a smaller mass number. During equilibrium reactions heavy isotopes tend to concentrate in the molecule where bond strengths are greatest. The structure and energy of two chemical species at equilibrium causes differences in their isotopic composition (a passive process). However isotope effects may occur during irreversible chemical reactions (an active process). The latter are usually termed kinetic isotope effects. The unreacted substrate becomes more enriched in the heavy isotope (^{13}C or ^{15}N) than the instantaneous product formed (Owens 1987, Hayes 1982).

In a single step irreversible reaction:

Substrate (S)	--->	Product (P)
(enriched)		(depleted)

There is discrimination against the heavy isotopes because they react more slowly than their light isotope counterparts. Kinetic fractionation effects are important for foodweb studies, since they occur when plants fix and assimilate C and N into organic compounds or when consumers (herbivores and carnivores) assimilate their food. Equilibrium effects are more relevant to the isotopic composition of carbon and nitrogen within the environment.

Isotopic fractionation is the basis for $\delta^{13}C$ and $\delta^{15}N$ variation in nature. There are two levels of variability. One is at the level of the whole organism (plant) and the other is at the level of its constituent biochemical components. To understand how the isotopic make-up of plants and animals within a foodweb vary, one requires a knowledge of the mechanisms whereby C and N are taken up by the primary producers at the base of the foodweb and those involved during assimilation by herbivores or carnivores.

C or N fixation (reduction to compounds utilisable by plants) generally involves depletion in ^{13}C or ^{15}N, so that plants have more negative $\delta^{13}C$ and $\delta^{15}N$ ratios than inorganic N and C containing compounds (Peterson & Fry 1987). Thereafter, however, consumers become isotopically enriched relative to their diet.

1.3.3 Carbon uptake by primary producers

In terrestrial plants, the initial fractionation is the results of an equilibrium isotope effect associated with CO_2 diffusion into the plant during photosynthesis (4.4 $^{o}/_{oo}$). The principle factors controlling $\delta^{13}C$ fractionation however, are photosynthetic pathway and the kinetic effects associated with CO_2 reduction by enzymes to form organic carbon-containing compounds utilisable by the plant. From here on, stepwise formation of metabolites takes place.

The C-3 pathway (Calvin-Benson pathway) is characterised by low $\delta^{13}C$ values (an average foliage value of -26.5 $^{o}/_{oo}$) (Smith & Epstein 1970; Vogel et al. 1978; van der Merwe 1982) compared to a value for atmospheric CO_2 of -7.7 $^{o}/_{oo}$ (Peterson & Fry 1987). This represents a depletion of about 20 $^{o}/_{oo}$. In photosynthesis, the first reaction of CO_2 reduction is a carboxylation reaction involving ribulose-1,5-biphosphate (or diphosphate, RuBP or RuDP), where CO_2 is converted to a three-carbon molecule, 3-phosphoroglyceric acid (PGA) (Zelitch 1979). RuBP discriminates against ^{13}C, causing ^{13}C depletion.

C-4 plants (Hatch-Slack pathway) have relativley more positive $\delta^{13}C$ values than C-3 plants, around -12.6 $^{o}/_{oo}$ (Smith & Epstein 1970; Vogel et al. 1978). The ß-carboxylase, PEPC (phosphoenolpyruvate carboxylase), catalyses the initial carboxylation and shows

little isotopic affinity (Zelitch 1979; Vogel 1980; Descolas-Gros & Fontugne 1990). CO_2 is reduced to a 4-carbon compound, oxaloacetate, which is then reduced to malate and/or aspartate in the chloroplasts (Zelitch 1979). After decarboxylation of malate, intracellular CO_2 is fixed again by RUBISCO.

CAM (Crassulacean Acid Metabolism) plants integrate these two pathways over a diel cycle and possess $\delta^{13}C$ values between the two (Teeri 1982).

Phytoplankton forms the base of pelagic foodwebs and are responsible for the assimilation of inorganic carbon. The $\delta^{13}C$ make-up of phytoplankton as POC (particulate organic carbon) from temperate shelf waters, ranges from ca. -18 $^{o}/_{oo}$ to -24 $^{o}/_{oo}$ with a mean of ca. -21 $^{o}/_{oo}$ (Fry & Sherr 1984). More positive and more negative $\delta^{13}C$ values are possible, probably depending on environmental conditions.

In the sea the initial fractionation is caused by an equilibrium isotope effect related to the relative proportions of dissolved CO_2 and HCO_3^- (bicarbonate) in the following equilibrium reaction (Deuser et al. 1968):

$$CO_2 + H_2O = H_2CO_3 = H^+ + HCO_3^-$$

Dissolved CO_2 is more negative (-9.2 $^{o}/_{oo}$ to -6.8 $^{o}/_{oo}$ from temperatures of 0 to 30 ^{o}C) than HCO_3^- (0 $^{o}/_{oo}$) due to differences in the bond strengths of these two molecular compounds (Deuser et al. 1968). Most of the inorganic carbon taken up by phytoplankton is in the form of bicarbonate (HCO_3^-) (Deuser et al. 1968), but there is a preference for CO_2 with increasing CO_2 concentration (Deuser et al. 1968). A drain on the availability of molecular CO_2 causes heavier CO_2 to be formed from the HCO_3^-. The greatest fractionation between bicarbonate and plant cells (the least ^{13}C discrimination and most depleted $\delta^{13}C$ value) occurs when CO_2 is most abundant (Degens et al. 1968a).

The $\delta^{13}C$ values for phytoplankton depend on both the carbon substrate taken up (CO_2 or HCO_3) and variations in the photosynthetic pathway used. Carbon fixation by RuBP occurs in marine phytoplankton (Descolas-Gros & Fontugne 1990). ß-carboxylases have since been found. These are phosphoenolpyruvate carboxylase, phosphoenolpyruvate

carboxykinase and pyruvate carbooxylase (Descolas-Gros Fontugne 1990). It appears that relatively high $\delta^{13}C$ values in phytoplankton are associated with high *in vitro* ß-carboxylase activities relative to RUBISCO activity, while lower $\delta^{13}C$ values are associated with high RUBISCO activity relative to that of ß-carboxylases. The rate of ß-carboxylation relative to RUBISCO activity can vary with environmental factors and physiological state of the cells. Factors such as temperature, PH and pCO_2, may affect the amount of dissolved CO_2 relative to HCO_3^- (Deuser et al. 1968; Fontugne & Duplessy 1978), and therefore, the $\delta^{13}C$ value of phytoplankton. Kinetic substrate —> product reactions may also be affected by changes in the environment.

1.3.3 a Temperature

Temperature dependence of plankton $\delta^{13}C$ values has been suggested but not proven. Isotopic theory predicts that in a substrate —> product reaction, isotopic depletion of the product formed occurs with an increase in temperature, due to increased mobility of the light isotope. However if the substrate is in limited supply, less discrimination against the heavy isotope will occur as the reaction proceeds. Examples of substrate —> product reactions that may be affected by an increase in temperature are rate of nutrient uptake and assimilation and, therefore, tissue turnover or growth rate. Hence, there may be a feedback mechanism governing the relationship between temperature and nutrient availability.

Fontugne & Duplessy (1981) found a positive correlation between $\delta^{13}C$ and temperature in phytoplankton. They calculated a change of ca. 0.35 $^{o}/_{oo}$ in phytoplankton per ^{o}C, consistent with the findings of Degens et al. (1968a), Christeller et al. (1976) and theoretical calculations by Libby (1972). Furthermore Rau et al. (1982, 1989) found a decrease in phytoplankton $\delta^{13}C$ from low to high latitudes which may be related to this relationship between $\delta^{13}C$ and temperature. Phytoplankton grown at low temperatures show an increase in RUBISCO concentration (Mortain-Bertrand et al. 1988), which may cause the associated depletion in ^{13}C. Fontugne & Duplessy (1978) suggested that temperature was not necessarily a direct influence. An increase in the proportion of lipid in plankton at lower temperatures may have added to the effect of ^{13}C depletion at low temperatures. Furthermore, phytoplankton from colder environments may be more depleted in ^{13}C than those from warmer areas due to the fact that CO_2 is more soluble in colder waters (Deuser et al. 1968). Deuser et al. (1968) found that the $\delta^{13}C$ difference

between phytoplankton and dissolved CO_2 remained the same in conditions of excess CO_2 (where CO_2 was bubbled into the system), in spite of a change in temperature. Hence another feedback mechanism is possible, that between temperature and CO_2 concentration, which may or may not be influenced by nutrient availability.

1.3.3 b Assimilation rate

Nitrogen limits zooplankton production (Checkley 1980). There exists a strong correlation between DIN (dissolved inorganic nitrogen) and the ratio of ß-carboxylase activities relative to RUBISCO activities in phytoplankton (Descolas-Gros & Fontugne 1990), with an associated increase in phytoplankton $\delta^{13}C$. ß-carboxylation gives the carbon skeleton for amino acid synthesis (Mortain-Bertrand 1988). Thus the concentration of inorganic nitrogen determines the rate of amino acid synthesis due to an increase in ß-carboxylase activities (Descolas-Gros & Fontugne 1990).

Checkley & Entzeroth (1985) found that zooplankton used nitrogen with greater efficiency than carbon. When primary productivity was increased by increasing the N supply, the $\delta^{13}C$ content of phytoplankton became more positive (Checkley & Entzeroth 1985), in contrast to an expected ^{13}C depletion due to preference for the faster reacting ^{12}C with an increase in assimilation rate. Nevertheless, the decrease in the availability of CO_2 during phytoplankton blooms may lead to a decrease in ^{13}C discrimination and/or increased assimilation of more HCO_3^- derived carbon, hence more positive $\delta^{13}C$ values.

1.3.3 c Age

High RUBISCO activities relative to ß-carboxylase activities during the initial exponential growth of skeletonema costatum, results in more negative $\delta^{13}C$ values than those during the terminal phase of exponential growth in cultured phytoplankton, which was associated with a high rate of organic carbon fixation by ß-carboxylation and a corresponding increase in $\delta^{13}C$ (Descolas-Gros & Fontugne 1985). In 1976 Pardue et al. observed maximum carbon fractionation (depletion relative to the inorganic carbon source) with low cell density or slow growth rate (Descolas-Gros & Fontugne 1990).

1.3.3 d pCO_2

The influence of pCO_2 on carbon isotope composition of plankton from warm water masses is small, but Descolas-Gros & Fontugne (1990) report that Calder & Parker (1973)

found that the $\delta^{13}C$ values of blue-green algae became more negative with increasing pCO_2, in contrast to the expected increase with increasing temperature.

1.3.3 e PH

At constant temperature the molecular CO_2 increases if the pH is lowered (Degens et al. 1968a). Therefore in conditions of low pH, one would expect phytoplankton to have more negative $\delta^{13}C$ values if the temperature remains constant.

1.3.3 f Photoperiod:

There is a strong interaction between light and RUBISCO activity (Descolas-Gros & Fontugne 1990). Furthermore, Descolas-Gros & Fontugne (1990) report that they have found a decrease in phytoplankton $\delta^{13}C$ associated with an increase of *in vitro* RUBISCO activity at the sea surface at midday (unpublished data).

Despite that environmental variables influence phytoplankton $\delta^{13}C$, species composition may also be an important controlling factor, as specific groups of marine phytoplankton may show significant differences in their carbon isotope ratio (Fry & Sherr 1984; Rau et al. 1982).

1.3.4 Nitrogen uptake by primary producers

Free nitrogen must be fixed i.e. incorporated in a chemical compound before it can be utilised by plants and animals. Nitrogen is fixed by some terrestrial and marine microorganisms (Delwiche 1970). Fixed nitrogen is also supplied by ionising phenomena such as cosmic radiation, meteor trails and lightning, which provide the energy for nitrogen to react with the oxygen or hydrogen of water. The supply of fixed nitrogen essentially involves the oxidation and reduction of nitrogen compounds, e.g. nitrification (the oxidation of ammonium, NH_4^+, to nitrite, NO_2^-, or nitrite to nitrate, NO_3^-) and denitrification (reduction of nitrites or nitrates to gaseous compounds such as N_2 and N_2O). Fixed inorganic N exists in the sea as dissolved NH_4^+ (highly reduced form) and NO_3^- (highly oxidised).

The principal factor controlling $\delta^{15}N$ in plants is the source of fixed nitrogen. Nitrification and denitrification in the sea both proceed with substantial isotope effects,

ranging in magnitude from 10 to 40 $^{o}/_{oo}$ (Mariotti et al. 1984; Owens 1985; Peterson & Fry 1987). The $\delta^{15}N$ in oceanic nitrate depends on geographic location, depth within the water column, and oxygen concentration. Nitrate dissolved in deep water has a $\delta^{15}N$ value of 6-8 $^{o}/_{oo}$ (Miyake & Wada 1967; Cline & Kaplan 1975; Wada & Hattori 1976). Data for SPM (suspended particulate matter) from various oceans (Pacific, Atlantic and Indian) have more positive $\delta^{15}N$ values on average, by ca. 6.5 $^{o}/_{oo}$ in deep water relative to shallow water (Checkley & Miller 1989). Nitrate $\delta^{15}N$ may reach 18 $^{o}/_{oo}$ in oxygen-depleted zones (Cline & Kaplan 1975).

1.3.4 a Nutrient supply

Isotopic theory predicts ^{15}N depletion in phytoplankton due to increased N-uptake with increasing nutrient supply. In the sea however, nitrogen supply often limits plant growth and enrichment in ^{15}N will occur when the nutrient is approaching exhaustion (following a period of nitrogen abundance). Where nitrogen is abundant, assimilation of NH_4^+ and NO_3^- by phytoplankton is indeed generally accompanied by ^{15}N depletion (Wada & Hattori 1978) only of ca. 4 - 6 $^{o}/_{oo}$ (Peterson & Fry 1987). Assimilation of NH_4^+ by phytoplankton may, however, lead to their enrichment in ^{15}N relative to their inorganic source (Mullin et al. 1984), but this is not always the case. The $\delta^{15}N$ of suspended organic matter has been found to increase with decreasing NO_3^- concentration (Wada & Hattori 1976). Hence one may expect an increase in phytoplankton $\delta^{15}N$ during a bloom as the available inorganic nitrogen supplies become exhausted (fractionation reduced as nitrogen becomes limiting). For example, Mariotti et al. (1984) demonstrated a strong seasonal variability in the ^{15}N content of SPM. Values for $\delta^{15}N$ varied from about +4 $^{o}/_{oo}$ prior to the Spring phytoplankton bloom in the Southern Bight of the North Sea, increasing to +10 $^{o}/_{oo}$ to +12 $^{o}/_{oo}$ during June and July at the height of the bloom, and declining during the autumn.

The $\delta^{13}C$ and $\delta^{15}N$ composition of an animal must equal the integrated isotopic composition of the carbon and nitrogen which is incorporated into the body, and that lost by excretion and/or respiration (De Niro & Epstein 1978). A large proportion of the nutrients utilised by heterotrophic bacteria and ultimately phytoplankton are provided by zooplankton excretory products (Azam et al. 1983). Checkley & Entzeroth (1985) found that zooplankton (Temora sp.) produce ammonium with a more negative $\delta^{15}N$ content than themselves, while faeces had more positive values relative to their bodies and

nitrogen source. Similarly for $\delta^{13}C$, respired CO_2 was more negative, while faeces were more positive relative to ingested material. The increase in copepod bodies and faeces was greater for $\delta^{15}N$ (+6 and +8 $^{o}/_{oo}$ respectively) than $\delta^{13}C$ (+2 and +1 $^{o}/_{oo}$ respectively). Thus towards the end of a phytoplankton bloom an isotopically more negative foodweb may be sustained by organisms recycling ammonia at the base of the foodweb (Checkley & Miller 1989). However, the presence of detritivores or the recycling of particulate matter by particle grazing zooplankton may result in their enrichment in ^{15}N and ^{13}C (particularly in ^{15}N).

1.3.4 b Light

Wada & Hattori (1978) found a decrease in $\delta^{15}N$ fractionation (greater depletion in ^{15}N relative to the inorganic nitrogen source) with decreased illumination.

1.3.4 c Growth rate

The growth rate of phytoplankton is inherently related to nutrient supply and light availability. Wada & Hattori (1978) found a strong relationship between $\delta^{15}N$ fractionation (usually depletion relative to the inorganic nitrogen source) and growth rate in light limited cultures.

1.3.4 d Age

Mariotti et al. (1982) showed that the isotopic fractionation associated with assimilatary NO_3^- reduction is not expressed in mature plants, implying a physiological basis for $\delta^{15}N$ fractionation with plant age.

1.3.4 e Temperature

An increase in temperature increases the reaction rate responsible for the supply of fixed nitrogen. (Mariotti et al. 1982; Wada & Hattori 1978). Hence nutrient supply, assimilation rate and ultimately growth rate, may sometimes be related to temperature. If nitrogen is not limited, isotopic theory predicts increasing ^{15}N depletion with increasing temperature.

1.3.5 Relationships with trophic level

In contrast to the isotopic depletion in phytoplankton relative to their source of inorganic carbon or nitrogen, whole animal $\delta^{13}C$ and $\delta^{15}N$ values reflect those of their diet (De Niro & Epstein 1978, 1981). Furthermore, a consumer tends to have more positive $\delta^{13}C$ and $\delta^{15}N$ values than its diet, with a larger diet-consumer difference for $\delta^{15}N$. The proportion of an organism's tissues that have $\delta^{13}C$ or $\delta^{15}N$ values more positive than their diet, e.g. muscle and collagen, is greater than the proportion made up of tissues more negative than the dietary source (Hare et al. 1991). It is important therefore, to distinguish the general trophic trend in isotope fractionation for whole animals with differing isotope abundance in different tissues.

Whole bodies of consumers are usually more enriched in ^{13}C than their diet by ca. 1-2 $^{o}/_{oo}$ (mostly < 1 $^{o}/_{oo}$ for marine organisms) (De Niro & Epstein 1978; McConnaughey & McRoy 1979; Macko et al. 1982; Thayer et al. 1983; Rau et al. 1983; Fry & Sherr 1984; Checkley & Entzeroth 1985; Kling & Fry 1992).

Consumers are usually more enriched than their diet by between 3 $^{o}/_{oo}$ and 5 $^{o}/_{oo}$ for $\delta^{15}N$ (Miyake & Wada 1967; De Niro & Epstein 1981; Macko et al. 1982; Minagawa & Wada 1984; Owens 1987). De Niro & Epstein (1981) and Minagawa & Wada (1984) report $\delta^{15}N$ diet-whole body relationships ranging from -0.5 $^{o}/_{oo}$ to +9.2 $^{o}/_{oo}$ (mean = 3.4 $^{o}/_{oo}$ ± 1.1 $^{o}/_{oo}$) and +1.3 $^{o}/_{oo}$ to +5.3 $^{o}/_{oo}$ (mean = 3.0 $^{o}/_{oo}$ ± 2.6 $^{o}/_{oo}$) respectively. Macko et al. (1982) found a strong species effect for the $\delta^{15}N$ relationship between the zooplankton and their food (Ulva and Gelidium sp.). Diet-consumer ^{15}N enrichments of -0.3 $^{o}/_{oo}$ and 2.3 $^{o}/_{oo}$ were found for Amphithoe valida and Parhyale hawaiensis, respectively.

The average relationships between whole bodies of animals and their diets are similar for a species regardless of diet (De Niro & Epstein 1978, 1981). However isotopic measurements of different individuals of a species can differ slightly (up to 1.8 $^{o}/_{oo}$ for $\delta^{13}C$ and up to 3.1 $^{o}/_{oo}$ for $\delta^{15}N$) (De Niro & Epstein 1981). The diet-consumer disparity for different species raised on the same diet may differ (De Niro & Epstein 1981).

Different planktonic consumers may show different magnitudes of isotopic enrichment, depending on the number of trophic interactions within the foodweb, and the proportion of phytoplankton in their diets (Kling & Fry 1992). Nevertheless, the correlation between more positive $\delta^{13}C$ and $\delta^{15}N$ values and position in a food web enables us to test the hypothesis that larger organisms will have more positive $\delta^{13}C$ and $\delta^{15}N$ values than smaller organisms in a pelagic food web. At the same time, we can investigate the isotopic differences between different fish tissues.

1.3.6 Variability in different biochemical components

The biosynthesis of amino acids and associated deamination and transamination processes, follows a complex pattern. As a result there are numerous ways that C can be arranged into various amino acids, hence different degrees of $\delta^{13}C$ and $\delta^{15}N$ fractionation in different biochemical components. The major biochemical fractions have characteristically different relative $\delta^{13}C$ and $\delta^{15}N$ values (Abelson & Hoering 1961; Park & Epstein 1961; Parker 1964; De Niro & Epstein 1978, 1981, Tieszen et al. 1983; Hare et al. 1991). Despite that the absolute values of particular amino acids within different consumers may differ, identical patterns of isotopic fractionation (relative to the amino acids in the diet) have been obtained across trophic levels (Hare et al. 1991).

Lipids have more negative $\delta^{13}C$ values relative to other biochemical components by up to 20 $^{o}/_{oo}$ (Park & Epstein 1961; Degens et al. 1968b; De Niro & Epstein 1978; Tieszen et al. 1983). De Niro & Epstein found the following relationships for $\delta^{13}C$, lipid < total organic matter, lipid < carbohydrate and lipid < protein. Tieszen et al. (1983) report the following relationship: lipid < liver < muscle < brain < hair. Lipids also have more negative $\delta^{13}C$ values than the total organism, but the difference is smaller (De Niro & Epstein 1978). The basic design of lipid synthesis is the same in all organisms (De Niro & Epstein 1977), essentially involving the formation of pyruvate from glucose, which is then decarboxylated and oxidised to acetyl coenzyme A (Embden-Meyerhof pathway). The ^{13}C depletion occurs during the formation of CoA from pyruvate due to a kinetic isotope effect associated with the formation of the pyruvate dehydrogenase complex (De Niro & Epstein 1977).

An animal's diet must contain the amino acids required for the amino acid composition of its tissues. If one of these amino acids is omitted from the diet, the animal will degrade tissue protein to meet its requirements and will go into negative nitrogen balance (Conn & Stumpf 1976). The importance of determining the isotopic composition of individual amino-acids when making assumptions about dietary interactions amongst organisms cannot be under-estimated.

1.3.7 Variability in muscle and bone collagen tissues

Muscle and bone collagen tissues are important since together they make up the largest mass of protein in the vertebrate body. The isotope ratios of carbon and nitrogen are fractionated to different degrees in these two tissues (De Niro & Epstein 1978, 1981; Lee-Thorp et al. 1989). The isotopic make-up of muscle and bone collagen tissues in an animal have relatively predictable relationships with its dietary source. Lee-Thorp et al. (1989) report muscle and bone tissues of terrestrial animals to be more enriched in ^{13}C than diet by ca. 2.5 $^{o}/_{oo}$ and 4.5 $^{o}/_{oo}$ respectively, where lipids were removed from the tissues. Hence bone collagen had a more positive $\delta^{13}C$ content than muscle tissue by 2-2.5 $^{o}/_{oo}$. These diet-tissue differences may be smaller if lipids are not removed from the tissues. In the case of $\delta^{15}N$ however, there is evidence that muscle tissue is more positive than bone collagen (De Niro & Epstein 1981). Muscle tissue from Mus musculus was more enriched in ^{15}N than the diet by 2.9 $^{o}/_{oo}$, whereas bone collagen from these mice fed the same artificial diet was enriched by only 2.6 $^{o}/_{oo}$.

Differences in the magnitudes of fractionation may be related to animal size. For example, Rau et al. (1981) found a strong positive correlation between the $\delta^{15}N$ values of muscle tissue from Dover Sole and increasing body weight. This may ultimately be due to differences in digestive physiology, or diet quality (Tiezsen 1988).

1.3.8 $\delta^{13}C$ and $\delta^{15}N$ values for pelagic organisms

Measurements of $\delta^{13}C$ values for marine organisms generally lie between -24 $^{o}/_{oo}$ and -10 $^{o}/_{oo}$ (Fry & Sherr 1984).

Phytoplankton from colder waters appear to have more negative $\delta^{13}C$ values than those from warmer waters. Data for phytoplankton as POC (particulate organic carbon) from temperate shelf waters range from ca. -18 $^{o}/_{oo}$ to -24 $^{o}/_{oo}$, with a mean of ca. -21 $^{o}/_{oo}$ (Fry & Sherr 1984). Mean values for POC from offshore foodwebs range from -25.3 $^{o}/_{oo}$ to 21.2 $^{o}/_{oo}$ (Fry & Sherr (1984). The data for POC from warmer offshore waters (Torres Strait, Straits of Malacca, Gulf of Mexico) lie between -12.8 $^{o}/_{oo}$ and -21.2 $^{o}/_{oo}$.

Although the $\delta^{13}C$ make-up of tropical and temperate phytoplankton is fairly constant around -21 $^{o}/_{oo}$, there is an increase in their $\delta^{13}C$ content during blooms, resulting in values between -16.8 $^{o}/_{oo}$ and -12.8 $^{o}/_{oo}$ for blue-green algae off Florida and in the Gulf of Mexico (Fry & Sherr 1984).

The $\delta^{13}C$ make-up of zooplankton largely depends on the $\delta^{13}C$ make-up of the phytoplankton on which they feed. Zooplankton tend to be more enriched than phytoplankton (Fry & Sherr 1984). Thayer et al. (1983) found total zooplankton or copepods to have more positive $\delta^{13}C$ values than phytoplankton by 0.8 to 2.2 $^{o}/_{oo}$ in the offshore Gulf of Mexico. Zooplankton in seagrass meadows range from -18 $^{o}/_{oo}$ to -16 $^{o}/_{oo}$ (Fry & Sherr 1987). Fry et al. (1984) report $\delta^{13}C$ values for zooplankton between ca. -18 and -20 $^{o}/_{oo}$, but they were more enriched by 2-4 $^{o}/_{oo}$ than POC at -24 $^{o}/_{oo}$ (Fry & Sherr 1984). Furthermore, water temperature is important since the $\delta^{13}C$ make-up of plankton may change considerably with temperatue (Fontugne & Duplessis 1981).

Values of $\delta^{13}C$ for fish in temperate oceans average more positive than -18 $^{o}/_{oo}$ (Rau et al. 1981; Chisholm et al. 1982; Rau et al.1983).

For $\delta^{15}N$, values for marine organisms range from ca. -2.1 to +14 $^{o}/_{oo}$ (Minagawa & Wada 1984, Sealy et al. 1987). However Schoeninger & De Niro (1984) concluded that $\delta^{15}N$ values for consumers in the marine environment range from +9.4 $^{o}/_{oo}$ to +25.0 $^{o}/_{oo}$, averaging +14.8 $^{o}/_{oo}$ to +23 $^{o}/_{oo}$ (n=61).

The $\delta^{15}N$ content of phytoplankton is likely to vary with environmental conditions affecting the $\delta^{15}N$ of NO_3^- and NH_3^+ and the concentration of NO_3^+. The majority of $\delta^{15}N$ values for SPM (suspended particulate matter) fall within the range, +6 $^{o}/_{oo}$ to +10 $^{o}/_{oo}$ (Altabet & McCarthy 1985). However, Miyake & Wada (1967) report more negative

$\delta^{15}N$ values for SPM to 3.2 $^{o}/_{oo}$ (excluding N-fixing species). Phytoplankton $\delta^{15}N$ values fall in the range 3 $^{o}/_{oo}$ to 12 $^{o}/_{oo}$ (Miyake & Wada 1967; Wada et al. 1975; Wada & Hattori 1976; Minagawa & Wada 1984; Sweeney & Kaplan 1980), close to the bulk of those found for SPM (hardly surprising since it is difficult to distinguish between the two components). However, very positive $\delta^{15}N$ values (ca. 20 $^{o}/_{oo}$ up to ca. 46 $^{o}/_{oo}$) have been measured for SPM at 100m depth from warm core rings (Altabet & McCarthy 1985). The $\delta^{15}N$ values for SPM generally increase with depth (Altabet & McCarthy 1985), as do the $\delta^{15}N$ values for marine algae (Wiencke & Fischer 1990). Furthermore, as for $\delta^{13}C$, phytoplankton may have more positive $\delta^{15}N$ values during a bloom, from 4 $^{o}/_{oo}$ to 10-12 $^{o}/_{oo}$ (Mariotti et al. 1984).

N_2-fixing phytoplankton (e.g. Trichodesmium) have been found to have more negative $\delta^{15}N$ values than non-N_2-fixing species (Wada et al. 1975; Wada & Hattori 1976; Minagawa & Wada 1984).

Zooplankton $\delta^{15}N$ is likely to vary in $\delta^{15}N$ with that of phytoplankton at the base of the foodweb. Nevertheless, zooplankton tend to be have more positive $\delta^{15}N$ values than phytoplankton and SPM (Miyake & Wada 1967; Wada et al. 1975; Wada & Hattori 1976; Minagawa & Wada 1984; Checkley & Entzeroth 1985). Minigawa & Wada (1984), report $\delta^{15}N$ values for zooplankton between 5.6 and 14.0 $^{o}/_{oo}$ from the Bering Sea. The $\delta^{15}N$ values for zooplankton from Lake Ashinoko fell within this range, from 6.8 to 9.4 $^{o}/_{oo}$ (mean = 8.1 $^{o}/_{oo}$). However, the $\delta^{15}N$ content of zooplankton associated with N-fixing phytoplankton were mostly more negative, reaching values close to 0.0 $^{o}/_{oo}$.

Minagawa & Wada (1984) found the $\delta^{15}N$ content of fish sp. in Lake Ashinoko to be around 11.1 $^{o}/_{oo}$ (10.7 to 11.6 $^{o}/_{oo}$) more positive than zooplankton from the same area.

1.4 PELAGIC ECOSYSTEMS OFF SOUTHERN AFRICA

1.4.1 Features of the southern Benguela ecosystem

The boundaries of the "Benguela current system (region)" are subjective interpretations of various combinations of meteorological, oceanographic, hydrodynamic and topographical

effects. Nevertheless, one may loosely describe the Benguela region as extending from about between 45°S and 35°S, the belt of the westerlies (Andrews and Hutchings 1980), to about 15°S, the northern boundary of the upwelling favourable wind field and the region of interaction with the southward moving Angolan Current (Shannon 1985). Its eastern boundary coincides with the Agulhas Current retroflection area south of Cape Agulhas (Shannon 1985; Shannon & Field 1985), but no attempt is made here to define its western boundary by such physical features. It is dominated by a coastal upwelling system, and a region of cool water along the west coast of South Africa. There is strong semi-permanent upwelling between about 25°S and 31°S, creating an effective environmental or thermal barrier between the northern Benguela and southern Benguela regions (Boyd & Cruickshank 1983; Shannon & Field 1985).

The region south of 31°S is characterised by a strong seasonal upwelling regime (Andrews & Hutchings 1980) extending from September to March (Andrews & Hutchings 1980). Spring and summer in the southern Benguela extend from about the end of September to the end of March (Andrews & Hutchings 1980). The prevailing winds over the Benguela region are determined by the South Atlantic high pressure system (anticyclone), the pressure field over the adjacent subcontinent and by eastward moving cyclones across the southern part produced by perturbations of the subtropical jet stream (Andrews & Hutchings 1980; Nelson & Hutchings 1983; Shannon 1985). The South Atlantic high is maintained throughout the year, but undergoes seasonal shifts in position from about 26°S : 10°E in Winter moving eastwards to about 30°S : 5°E in Summer (Andrews & Hutchings 1980; Shannon 1985). The summer position of the pressure systems favours a predominantly southerly wind regime over the entire area, peak frequencies occurring in spring and late summer (Andrews & Hutchings 1980). The southerlies cause movement of surface water away from the west coast, favouring upwelling of cold, nutrient rich, deeper lying water. Thus a semi-permanent plume of cold water runs from the Cape peninsula, in a north-westerly direction, throughout the summer months, isolated from South Atlantic Surface Water by a well defined front due to a strong temperature and salinity gradient (Shannon 1985; Andrews & Hutchings 1980). The front varies in position and intensity with wind strength and intensity (Andrews & Hutchings 1980), but coincides with the run of the shelf break (Shannon 1985). The front is associated with a powerful north flowing jet current extending to 200m (Andrews & Hutchings 1980). In winter, the northward shift of the pressure systems causes an increase in the frequency of westerly

winds, not favourable for upwelling, but favouring a predominance of downwelling from about April to September, in the region of the southern Benguela (Andrews & Hutchings 1980; Shannon 1985). Primary productivity is therefore high during Spring and Summer, but low during Autumn and Winter (Andrews & Hutchings 1980). During the upwelling season wind relaxation or reversals causes upwelling in the southern Benguela to be modulated on the time scale of about one week (Nelson & Hutchings 1983). The most intense upwelling tends to occur off the central part of the Peninsula (Andrews & Hutchings 1980).

In summer, upwelling can extend as far south and east as Cape Agulhas (35°S : 20°E), which may be regarded as the appropriate western boundary of the southern Benguela system and an integral part of the productive western coast regime (Shannon 1985). Here, the influence of the Agulhas Current causes water temperatures to increase (Shannon 1985; Crawford 1980). This is an important spawning ground for commercially important fish species, the warmer water favouring the rate of egg development (Crawford 1980).

The upwelled water off the west coast of South Africa is of South Atlantic Central Water origin (Andrews & Hutchings 1980). South Atlantic, South Indian subtropical surface water and Agulhas current water, present offshore in the south, is advected in eddies northwards around the Cape during the upwelling season (Shannon 1985). It is difficult to assess the degree of interaction between the Benguela and Agulhas current systems on the basis of their thermohaline characteristics. Therefore, it equally difficult to assess their influence on the isotopic composition of organisms associated with these eddies, particularly larger organisms which reflect a longer dietary history.

Mean monthly sea surface temperature measurements show a clear seasonal variability in the Benguela (Brown & Field 1986; Taunton-Clark & Shannon 1988; Taunton-Clark & Kamstra 1988). In summer, inshore waters decrease in temperature so that a 10-11°C difference is sometimes found between inshore and offshore surface waters (Andrews & Hutchings 1980). In winter, inshore and offshore surface water is of rather uniform temperature. SST during the upwelling season ranges from 9.0 to 17.7°C (Brown & Field 1986). Monthly variability in the southern Benguela is high in the upwelling areas (Brown & Field 1986; Taunton Clark & Shannon 1988) and substantial changes (up to 6°C) can occur in 24 h (Brown & Field 1986). Temperatures during winter are more consistent

(Brown & Field 1986; Taunton-Clark & Shannon 1988) ranging from 12.8 to 13.6°C (Brown & Field 1986). Brown & Field (1986) and Andrews & Hutchings (1980) observed that winter temperatures are on average slightly higher than summer temperatures. However, Taunton-Clark & Shannon (1988) found that mean monthly summer SST (sea surface temperature) anomalies in the upwelling region of the southern Benguela for the period 1906-1984, were slightly warmer than winter, peaking during November, while the coldest months were around July. The amplitude due to seasonally related shifts of SST was only 3°C.

It seems probable, from the biological record, that decadel cyclical perturbations of sea temperature occur in the Benguela (Walker et al. 1984; Taunton-Clark & Kamstra 1988; Taunton-Clark & Shannon 1988; Shannon & Agenbag 1990; Shannon 1985). Related environmental indices such as atmospheric pressure, winds, sea level and Trenberth's Southern Oscillation Index have also been shown to indicate periodicity on similar time scales (Taunton-Clark & Kamstra 1988). Nevertheless, this large scale interannual variability in the southern Benguela is small and insignificant compared with the seasonal oscillations. For example, a 2°C SST anomaly in spring of 1963 was the only statistically significant deviation at the 95% confidence level computed for any seasonal value for a 26 year period (1958-1981) (Walker et al. 1984).

Data from cruises monitoring the 1969-70, 1970-71, 1971-72 and 1972-73 upwelling seasons, reveal the existence of trends in the relationships between temperature and the nutrient, chlorophyll and oxygen concentrations (Andrews & Hutchings 1980). The cold upwelling water (around 9°C) is associated with low chlorophyll (from about 0.4 to 0.9 $\mu g\ l^{-1}$) and low oxygen (around 4.4 ml l^{-1}) conditions, but is high in silicates (15-16 $\mu g\ l^{-1}$), nitrates (18-24 $\mu g\ l^{-1}$) and phosphates (around 1.5 $\mu g\ l^{-1}$). Silicate, nitrate and phosphate concentrations steadily decrease with increasing temperature, oxygen concentrations steadily increase, while chlorophyll concentrations peak at intermediate temperatures. During the upwelling season, modulations in upwelling intensity (Shannon 1985), can cause considerable day to day variation in the horizontal distribution of nutrients, chlorophyll and oxygen (Andrews & Hutchings 1980). The work of Probyn (1985) suggests that net plankton productivity is largely nitrate controlled, but nanoplankton productivity is largely based on regenerated nitrogen.

According to Shannon and Field (1985), the productively active area of the southern Benguela region stretches from the Olifants River on the west coast to Cape Agulhas on the south coast, an area covering about 40 000 km^2. Diatoms form the bulk of the phytoplankton in the southern Benguela (Shannon & Pillar 1986). Dinoflagellates share this apparent dominance to some degree (Shannon & Pillar 1986). However our understanding of important phytoplankton species may be biased due to past inadequacies in sampling techniques. Nanoplankton may form relatively stable and persistent populations becoming increasingly important under conditions unfavourable for diatom growth (Shannon & Pillar 1986). Probyn (1985) found that picoplankton (<1 μm) and nanoplankton (<10 μm) chlorophyll a accounted for 2-49% and 13-99% respectively of the whole community chlorophyll-a during December 1983.

The southern Benguela region supports a phytoplankton standing stock of about 10 - 11 g Cm^{-2} dry weight (Andrews & Hutchings 1980; Moloney & Field 1985; Shannon & Field 1985). The maximum phytoplankton standing stock calculated by Shannon & Field (1985) was 700 000 tonnes C in the southern Benguela during May 1978. However, the standing stock was less in late winter (minimum during August was around 256 000 tonnes C). Andrews & Hutchings (1980) also found the standing stock of phytoplankton to be considerably higher during the upwelling season. Wind reversals result in inshore-offshore fluctuations of phytoplankton and zooplankton stocks along the Cape Peninsula upwelling plume during summer. Strong upwelling displaces the phytoplankton out of the area, but during the intermittent quiescent periods high production and high concentrations occur as a result of increased nutrient levels (Andrews & Hutchings 1980). Nutrient depletion restricts summer phytoplankton growth and causes negative gross production in surface waters. The resulting decomposition due to nitrate depletion is made available to subsurface plankton. In Autumn, negative production may also occur below 20m from decreasing light levels (Andrews & Hutchings 1980).

Despite the fact that zooplankton numbers increase during spring and summer in response to increased productivity, the zooplankton standing stock is only a fraction of the phytoplankton standing stock and daily production (Andrews & Hutchings 1980; Shannon & Field 1985), and does not materially influence phytoplankton stocks (Shannon & Pillar 1986). Andrews & Hutchings (1980) found that the mean zooplankton standing stock in summer was higher than in winter with a summer maximum (10 g m^{-2} dry

weight) about 2-3 times the winter minimum (mean = 2.3 g m^{-2}). A large proportion of the zooplankton population was found to remain above the thermocline during daylight hours in the upwelling plume and did not undergo extensive vertical migrations across the thermocline.

The mean phytoplankton standing stock is capable of supporting a total pelagic fish production of about 2 million tonnes wet (117650 tonnes dry) (Shannon & Field 1985). Taking the annual carbon requirements of pelagic fish in the southern Benguela to be about 3 million tonnes, and assuming that about two-thirds of their diet is made up of phytoplankton (one-third zooplankton), the phytoplankton biomass is near to its carrying capacity for the region (Shannon & Field 1985).

The total Cape pelagic catch fluctuates around 400 000 tonnes (Shannon & Field 1985). Pilchards comprised the bulk of the catch prior to 1965. During the 60s, the pilchard population suffered a massive decline, replaced by anchovy and to a lesser extent, roundherring. It is believed that the collapse of the pilchard stock was at least partly caused by overfishing.

1.4.2 The region of the Agulhas Bank

Shannon & Pillar (1986) described the nearshore region between Cape Point and Danger Point as the western Agulhas Bank. In this study, I refer to the whole area east of Cape Point as the Agulhas Bank. However, the Agulhas Bank is usually described as that region extending from Cape Agulhas to Cape St Francis (Fig. 4), and is assumed to lie within the 200m depth contour (Moloney & Field 1985). The two areas (west and east of Cape Agulhas) are normally distinct from one another in terms of physical features and processes, but together are an important spawning area for many pelagic fish species (Crawford 1980). The Agulhas Bank area (excluding the region west of Danger Point) covers about 50 000 km^2.

The Agulhas Bank is influenced by the warm Agulhas Current (20-26^{o}C). There is no summer upwelling regime, but local upwellings occur periodically as a result of a combination of coastal topography and wind direction. The warmest time of the year

coincides with the period of maximum insolation (summer). Mean monthly SST estimates for the years 1906-1986 show a peak around January at ca. 21°C, mostly >18 °C from November to March (data from Taunton-Clark & Shannon 1988). There is a greater temperature difference between summer and winter months than occurs in the southern Benguela upwelling region, with mean SST values reaching ca. 16°C in July-August (5°C amplitude). However, throughout the year, the Agulhas Bank is warmer on average than the southern Benguela, particularly during summer.

The average standing stock of phytoplankton in the Agulhas Bank region has been estimated to be 216 000 tonnes (Moloney & Field 1985). Shannon & Field (1985) calculated a daily production rate of 1.5 g C m^{-2} d^{-1} for phytoplankton in the spawning area. Thus the phytoplankton standing stock in this region is estimated to be about ≤ half that of the southern Benguela region (data from Andrews & Hutchings 1980; Moloney & Field 1985; Shannon & Field 1985). In these waters growth of phytoplankton is frequently restricted to the region of the thermocline, with a subsurface maximum during summer (Crawford 1980; Hutchings pers comm.). However, in winter, there is a more uniform distribution. Winter storm mixing to the bottom and relatively high chlorophyll a concentrations in the upper 40m, suggests nutrient supply by sediments (Shannon et al. 1984).

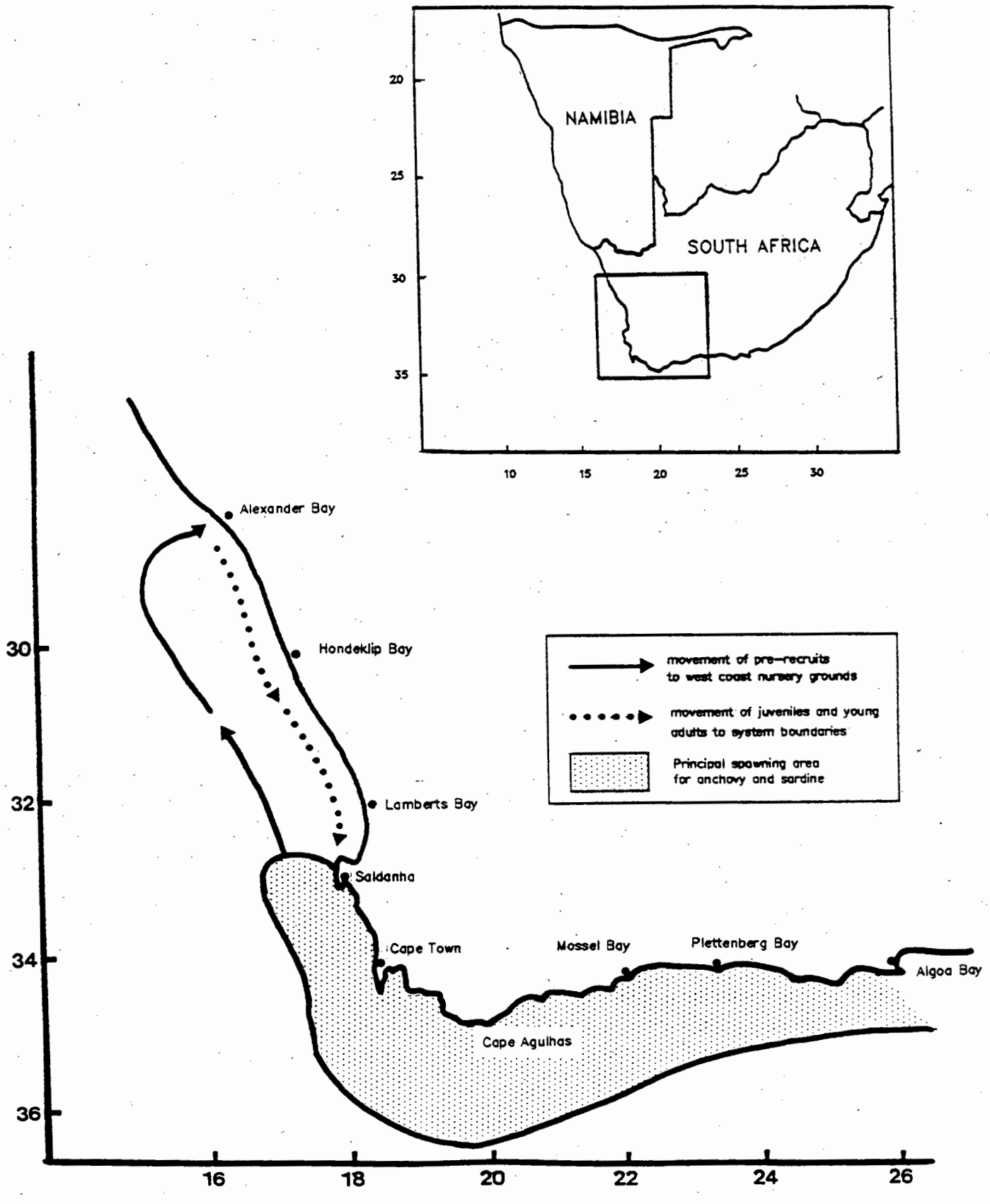
The biomass of copepods and anchovies across the Agulhas Bank in summer appears to be inversely related (Peterson et al. 1991). In 1988, for example, anchovies were most abundant on the western Bank (10 mg m^{-2} dry weight), but there was virtually nothing in the water column for them to eat. Copepod biomass in the same area was around 0.5 mg m^{-2} (dry weight) which is the lower threshold concentration at which anchovies will begin feeding (James 1988). Furthermore, only a small percentage of this would be available for consumption by the fish in terms of prey size and their spatial variability. Copepod production rates were much higher on the eastern than the western Agulhas Bank, together with higher primary production and chlorophyll concentrations. Copepod biomass on the eastern Bank was about 4-6 mg m^{-2}, 8-12 times as much as that in the western region, but anchovy biomass was lower by about the same order of magnitude.

Pilchard and anchovy are preyed upon extensively by Hake, Merluccius spp., in the eastern area during May and June, but are infrequently observed (Crawford 1980). Thus it seems likely that they occur mostly below the thermocline.

1.4.3 Diet and migratory patterns of pelagic organisms off the coast of southern Africa

The most important pelagic fish species in terms of abundance in the commercial catches are anchovy, Engraulis capensis, pilchard, Sardinops ocellatus and Cape horse mackerel, Trachurus trachurus (Moloney & Field 1985). Roundherring, Etrumeus whiteheadi, is also one of the dominant species in the southern Benguela and all four species together fall into the category of spring/summer spawners (Crawford 1980). Anchovy and sardine spawn in the region of the Agulhas Bank, east of Cape Point (Crawford 1980). Shelton and Hutchings (1982) have demonstrated the importance of the Good Hope Jet (Bang and Andrews 1974) in the northward transportation of anchovy eggs and larvae along the west coast. Other species may utilise the same mechanism. Recruitment of nought year olds of these spring/summer spawners takes place along the west coast from mid-autumn onwards (Fig. 1 shows the spawning and recruitment areas for anchovy and sardine within the study region). With the onset of spring there is a tendency for them to move south into regions of newly initiated upwelling. One year old pilchard, ca. 150 mm caudal length (Waldron et al. 1989), and roundherring (no length-age correlations) are generally not reproductively active and remain on the west coast. At the end of their first year, anchovy are about 100 mm caudal length (Waldron et al. 1989), and are sexually mature. With the onset of spring, they migrate with older anchovy and pilchard southwards, and to the east of Cape Point to spawn. Anchovy spawning is largely confined to the period October to January (Crawford 1980). About 50% of two-year-old sardine, together with three and four year old sardine, occur in the southern spawning region during spring and summer. Most sardine spawning takes place between September and February (Crawford 1980). Two-year-old pilchard dominate the winter "sardine runs" off the Transkei and Natal coasts, contributing little to the western Cape fishery (Baird 1971). Five and six group pilchard, prior to their decline in abundance during the 1960's, were regular spawners offshore of St. Helena Bay, probably in the warmer waters outside the oceanic front (Crawford 1980).

FIG. 1 Map showing anchovy, Engraulis capensis and sardine, Sardinops ocellatus, spawning and recruitment areas within the southern Benguela ecosystem off South Africa (adapted from Crawford et al. 1987).



It is difficult to examine the preferred food source for pelagic fish since they have the ability to forage over three "trophic levels" (phytoplankton, herbivorous zooplankton and carnivorous zooplankton). Indeed, a historical synopsis shows considerable debate over the preferred diets of intermediate microphagist clupeids (James 1988). Nevertheless there are physical (e.g. gill raker morphology; King & Macleod 1976), physiological (e.g. enzyme activity and morphology of the alimentary tract) and energetic (e.g. increased oxygen consumption and drag associated with filter feeding; Durbin et al. 1981) constraints on particular feeding strategies for each species. For positive growth, the minimum requirements of prey size, plankton concentration and foraging time must be exceeded (James 1988). The most efficient mode of feeding may be considered the primary feeding strategy, and the food type associated with that strategy, the primary food source.

The Cape anchovy is the mainstay of the South African and Namibian purse seine fisheries, with a combined total annual catch exceeding 600 000 tonnes (James 1988). James (1987) found E. capensis to be a selective forager on "mesozooplankton" (0.7mm to 20mm). Smaller prey which elicited filter feeding, caused large increases in swimming speed with little effect on clearance rate, thus were energetically expensive. Calanoid copepods and euphausiids constituted the bulk of the diet and were selected mainly on the basis of size. The phytoplankton component of the diet of anchovy were mainly diatoms, especially Coscinodiscus, which may form dense blooms in the St. Helena Bay region during autumn (Shannon & Pillar 1986). However, (James 1988) showed that foraging time was important for determining the efficiency of filter feeding for anchovy. A shorter foraging time was required for the same energetic benefit if filtering on larger than smaller prey.

Gut content analyses indicate that E. whiteheadi is largely a macrozooplanktivore, the bulk of the diet consisting of copepods, euphausiids and decapods (Wallace-Fincham 1987). S. ocellatus appears to filter feed more than these other two species. (Davies 1957), using the points method, concluded that these fish were phytophagous with a low percentage of zooplankton in the diet (70% phytoplankton made up of 21% diatoms, 4% dinoflagellates, 45% unrecognizable). Cushing (1978) suggested that Davies (1957) underestimated the importance of zooplankton volume in the diet of this fish. Furthermore, Loukashkin (1970) found no preference for either phytoplankton or zooplankton by these fish. Based on numerical counts, King & MacLeod (1976) observed

that both anchovy and pilchard switch their diet from zoophagy to phytophagy at about the 80mm and 100mm stage (standard length) respectively. They attributed this change in diet to the completed development of the gill raker system, enabling these fish to consume a "preferred" diet of phytoplankton.

CHAPTER 2

THE INFLUENCE OF LIPID CONTENT ON CARBON ISOTOPE COMPOSITION

INTRODUCTION

The more negative $\delta^{13}C$ values for lipids relative to other biochemical fractions may result in significantly more negative $\delta^{13}C$ values for the total organism (see section 1.3.3.). Variations in the lipid content of an organism due to differences in age, reproductive status, sex, species, season, water temperature, etc., may affect its $\delta^{13}C$ value. In this thesis therefore, lipids were removed from the samples before isotopic measurement. This has not been done in several other studies. In order to make the data comparable, it is necessary to determine what effect lipid removal has on $\delta^{13}C$ determinations in different pelagic organisms.

MATERIALS AND METHODS

Different size classes of plankton, anchovy (Engraulis capensis), sardine (Sardinops ocellatus) and Cape hake (Merluccius capensis), were used. Relationships between lipid content and organism size could therefore be examined.

Sampling

- Plankton

Samples of plankton were collected from the Agulhas Bank during a cruise aboard the R.V. Meiring Naude in November 1989 (see Fig. 6). Sampling was carried out with vertical hauls using a BONGO net (200μm mesh diameter) and oblique tows using a 300 μm mesh diameter neuston net. The samples used in this study were caught above the thermocline (ca. 50-70m). The samples were screened on board into five size classes (<200 μm, 200-500 μm, 500-1600 μm, 1600-3500 μm and >3500 μm), by washing fresh samples through screens aboard ship with seawater. Only the 200-500 μm and 500-1600 μm size-classes (the bulk of the plankton) were used for the experiments in this chapter.

- Fish

Samples of anchovy and pilchard were caught south and east of Cape Point during a cruise aboard the RV Africana in November 1989, using an Engels 308 midwater trawl. Fish from each trawl were sorted into 10mm size classes. Samples of hake were provided by Colleen Parkins and were included in the experiment to compare the results with larger fish, further up the pelagic foodweb. The hake were captured from the west coast off Cape Columbine.

Muscle tissue was removed from the dorso-anterior end of each fish and divided into two samples, one used for lipid extraction. For plankton, whole individuals from the same trawl and size-class were used for each comparison. A number of animals were accumulated to provide an optimum sample size of ca. 10mg.

Sample preparation

In each case, lipids were removed from one member of the paired samples in a solution of chloroform, methanol and water (2:1:0.8), by the method of Bligh & Dyer (1959).

Stable isotope analysis

A sample of 10-15 mg was weighed into a quartz breakseal tube with excess copper, copper oxide and silver foil. The tube was evacuated to 10^{-2} Torr, sealed, and combusted in a furnace at 800°C for eight hours. The resultant CO_2 and N_2 gases were separated on a vacuum line in the same way as described by Lanham (1983) and Handley et al. (1991). Isotope ratios were measured using a VG Micromass 602E 90° sector double-collector mass spectrometer. Isotope ratios were calculated as:

$$\delta X = \{(R_{sample} - R_{std})/R_{std}\} \times 1000 \ (^{o}/_{oo}),$$

where X = ^{13}C, R = $^{13}C/^{12}C$, and std = Peedee Belemnite carbonate (PDB) (De Niro & Schoeninger 1983, Rau et al. 1989). The measurement error (standard deviation) for homogeneous sample materials is less than 0.08 $^{o}/_{oo}$ (present data). N_2 gases produced during the combustion process were pumped away, since lipid (CHO) removal should not affect the $\delta^{15}N$ make-up of organisms. However, these may be captured using activated charcoal and $\delta^{15}N$ values determined as above. In this case, X = ^{15}N, R = $^{15}N/^{14}N$ and std = atmospheric (AIR) nitrogen for $\delta^{15}N$ (De Niro & Schoeninger 1983, Rau et al. 1989).

Wilcoxon paired sample tests (Zar 1984) were used to test for significant differences between lipid-free and untreated samples (Table 2). Kruskal-Wallis one-way ANOVA was performed on the differences for plankton and anchovy, sardine and hake muscle tissues (Zar 1984). Spearman's rank correlations were carried out to determine the relationships with fish length (Zar 1984). Lord's range tests were used to determine the differences between small and large plankton (Langley 1968).

RESULTS AND DISCUSSION

Table 1a shows the relationship between the $\delta^{13}C$ values for both untreated plankton samples and those from which lipids were removed (n = 4). The samples are in the 200-500 μm and 500-1600 μm size-fractions. The untreated samples have significantly more negative $\delta^{13}C$ values than the defatted samples (Table 2), by up to 2.3 $^{o}/_{oo}$. Plankton in the larger 500-1600 μm size-class show more ^{13}C depletion due to lipids than those in the 200-500 μm size-class (not significant). Furthermore, both untreated and defatted samples in the larger 500-1600 μm size-class have more positive $\delta^{13}C$ values than those in the 200-500 μm size-class (not significant).

Table 1b shows the relationship between the $\delta^{13}C$ values for untreated and lipid free anchovy muscle samples of fish between 80 mm and 130 mm caudal length (n = 4, each fish a different size-class). Once again the untreated samples have significantly more negative $\delta^{13}C$ values than the defatted samples (Table 2), by up to 1.2 $^{o}/_{oo}$. Except for the 8-9 cm size-class, there is a suggestion that the difference between the untreated and defatted samples of muscle tissue from larger fish is greater, but this is not significant. Given more data, it is possible that isotopic trends with organism size may be affected where such differences between untreated and defatted samples appear also to be related to organism size. Both untreated and defatted muscle samples show a trend whereby anchovy have more positive $\delta^{13}C$ values with increasing fish size, but this is not significant.

Table 1c shows the relationship between the $\delta^{13}C$ values for lipid free and untreated muscle samples from sardine between 120 mm and 210 mm caudal length (n = 4, each fish a different size-class). For these few sardine there is a $\delta^{13}C$ increase of 1.1 $^{o}/_{oo}$ with

TABLE 1: The $\delta^{13}C$ results for untreated plankton samples versus those from which lipids were removed. S.D. = standard deviation, Defat. = results for samples from which lipids were removed, Not = results for untreated samples, difference = the difference between the treated and untreated samples.

a) PLANKTON

Sample	Size	$\delta^{13}C$ Defat.	Not	difference
1	200-500μm	-18.4	-19.7	1.3
2	200-500μm	-17.7	-19.4	1.7
3	500-1600μm	-16.1	-19.2	3.1
4	500-1600μm	-17.0	-19.3	2.3
	mean (S.D)			2.1 (0.7)

b) ANCHOVY

Sample	Size	$\delta^{13}C$ Defat.	Not	difference
1	8-9cm	-16.0	-17.2	1.2
2	10-11cm	-17.3	-17.5	0.2
3	11-12cm	-15.6	-16.4	0.7
4	12-13cm	-15.7	-16.6	0.9
	mean (S.D.)			0.8 (0.4)

c) SARDINE

Sample	Size	$\delta^{13}C$ Defat.	Not	difference
1	12-13cm	-16.2	-16.6	0.4
2	15-16cm	-15.5	-15.8	0.3
3	16-17cm	-15.3	-15.9	0.6
4	20-21cm	-15.1	-15.5	0.5
	mean (S.D.)			0.5 (0.1)

d) HAKE

Sample	Size	$\delta^{13}C$ Defat.	Not	difference
1	17cm	-15.4	-16.0	0.6
2	27cm	-15.3	-16.0	0.7
3	40cm	-15.0	-15.2	0.2
4	62cm	-15.0	-15.3	0.3
5	80cm	-14.0	-14.7	0.7
	mean (S.D.)			0.5 (0.2)

increasing fish length for both untreated and lipid free samples (not significant). The differences between the untreated and defatted samples are significant (Table 2), but show no relationship with fish length. The largest difference is 0.6 $^{o}/_{oo}$, less than for anchovy and plankton.

Table 1d shows the $\delta^{13}C$ data for both untreated and lipid free samples of hake muscle, representing fish from 170 mm to 800 mm caudal length (n = 5, each fish a different size-class). In this case, the data for the lipid free muscle tissue was obtained from Colleen Parkins (U.C.T. Zoology Honours project). In all cases the untreated samples have significantly more negative $\delta^{13}C$ values than the defatted samples (Table 2), by up to 0.7 $^{o}/_{oo}$. There is an increase in $\delta^{13}C$ of ca. 1.4 $^{o}/_{oo}$ with increasing fish length for both untreated (not significant) and lipid free ($R_S = 0.97$, df = 4, $p < 0.05$) samples.

SUMMARY & CONCLUSIONS

It appears that lipids cause $\delta^{13}C$ values to be more negative for all the plankton and fish measured here. Kruskal-Wallis one-way ANOVA reveals that plankton show more lipid-related depletion in ^{13}C than do fish, and of the fish, anchovy show the most $\delta^{13}C$ depletion due to lipids ($H = 9.86$, $p < 0.02$). Within the plankton and anchovy, it appears that larger organisms show more lipid-related depletion than smaller organisms.

Considering that the $\delta^{13}C$ difference between a consumer and its diet is usually < 1 $^{o}/_{oo}$, the magnitudes of lipid-related depletion in ^{13}C shown by the samples in this study have important implications for foodweb studies. This is particularly important in the case of plankton, where $\delta^{13}C$ differences between different size classes of plankton are of the order of 0.3 to 1.4 $^{o}/_{oo}$ (Kling & Fry 1992; Sholto-Douglas et al. 1991).

Although lipids may complicate the interpretation of foodwebs, if one is interested in the isotopic behaviour of all the biochemical fractions from diet to consumer, it may be useful to measure the $\delta^{13}C$ values and concentration of the lipid fraction removed.

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Table 1d shows the $\delta^{13}C$ data for both untreated and lipid free samples of hake muscle, representing fish from 170 mm to 800 mm caudal length (n = 5, each fish a different size-class). In this case, the data for the lipid free muscle tissue was obtained from Colleen Parkins (U.C.T. Zoology Honours project). In all cases the untreated samples have significantly more negative $\delta^{13}C$ values than the defatted samples (Table 2), by up to 0.7 $^{o}/_{oo}$. There is an increase in $\delta^{13}C$ of ca. 1.4 $^{o}/_{oo}$ with increasing fish length for both untreated (not significant) and lipid free (R_S = 0.97, df = 4, $p < 0.05$) samples.

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Although lipids may complicate the interpretation of foodwebs, if one is interested in the isotopic behaviour of all the biochemical fractions from diet to consumer, it may be useful to measure the $\delta^{13}C$ values and concentration of the lipid fraction removed.

CHAPTER 3

TISSUE TURNOVER IN ADULT SARDINE, SARDINOPS OCELLATUS

INTRODUCTION

It is important to measure how quickly a change in diet effects the isotope composition of the tissues of pelagic fish since dietary changes may occur with abundance, size and availability of prey (Koslow 1981; Angelescu 1982; James 1987; James & Findlay 1989) and at at certain stages of a fish's life cycle (King & Macleod 1976). Larger organisms have a slower metabolic and tissue turnover rate than do smaller organisms. Hence, there is a longer dietary integration period for larger organisms, and we can expect pelagic fish to be less isotopically sensitive to changes in diet than shorter-lived planktonic organisms.

MATERIALS & METHODS

Adult sardine were caught in August 1988 from the region of St. Helena Bay, off the west coast of South Africa, and maintained in a circular pool, ca. 1m deep and ca. 1.5m diameter. Fresh seawater was continually replaced from the sea adjacent to the institute, at a rate of ca. 4.0 l. min^{-1}. A period of 14 months elapsed before this experiment was initiated (the beginning of November 1989). During this time, the fish were fed a diet of commercial trout pellets (mean $\delta^{13}C$ = -18.54, mean $\delta^{15}N$ = 11. 39, n=3 in both cases). To determine the average isotopic composition of the fish on the diet of trout pellets, 5 fish were sacrificed over a period of time for isotope analyses. After an initial sampling interval of one week (to determine the rate of change in the isotopic make-up of the fish tissues), one fish a month was sacrificed for isotope analysis (see Appendix 1). After 13 weeks, the diet was changed to one of snoek (Thyrsites atun) roe (mean $\delta^{13}C$ = -16.3, mean $\delta^{15}N$ = 15.4, n=3 in both cases). No sampling was carried out for the next 8 weeks, allowing for a period of isotopic integration. The sampling regime was then resumed for another 14 weeks, initially using a weekly sampling interval, followed by a monthly interval. The total duration of the experiment was 35 weeks (to the beginning of July 1990).

Sample preparation

Muscle tissue and vertebrae were removed from each fish. Initially, different parts of fish muscle were measured in order to establish the degree of variation within an individual. The $\delta^{13}C$ content of red muscle from the tail area of these sardine, was more negative than white muscle tissue, from the anterior part of the fish, by up to 2.3 $^{o}/_{oo}$ (mean = 1.5 $^{o}/_{oo}$), but the $\delta^{15}N$ content of red muscle was slightly more positive than white muscle, by up to 0.8 $^{o}/_{oo}$ (mean = 0.6 $^{o}/_{oo}$). It was decided to use muscle from the anterior part fish throughout the experiment to reduce variation associated with different muscle types from different parts of the body. White muscle was chosen to gain a larger dietary-consumer enrichment factor for carbon. Furthermore, a better estimate of total turnover would be obtained, since white, glycolytic, muscle fibres have a slower rate of turnover (see section 1.2).

Samples were defatted (as described in chapter 2) to minimise discrepancies resulting from differences in fat tissue proportions between different organisms. Bone samples were decalcified in 1.5% hydrochloric acid (Sealy et al. 1987) leaving the collagen for analysis. Samples were then freeze dried for 24 hours and stable isotope analyses carried out as described in chapter 2. The data are shown in Appendix 1.

Furthermore, the alimentary canal posterior to the gut was removed from 5 fish fed the diet of trout pellets, and 2 fish fed snoek roe. Their contents were removed, defatted, and the $\delta^{13}C$ and $\delta^{15}N$ values measured.

Spearman's rank correlation coefficients (Zar 1984) were used to measure the change in $\delta^{15}N$ within the tissues of sardine due to a change in diet.

RESULTS AND DISCUSSION

Figs. 2 to 5 show the $\delta^{13}C$ and $\delta^{15}N$ data for sardine muscle and bone collagen tissues during the experiments in which their diet was changed from commercial trout pellets to snoek roe. The $\delta^{13}C$ data for the sardine muscle tissue (Fig. 2) show no clear response to the change in diet to snoek roe. The last two samples however, had the most negative $\delta^{13}C$ values. The mean $\delta^{13}C$ value for the muscle of sardine fed trout pellets (-14.1 $^{o}/_{oo}$) was 4.4 $^{o}/_{oo}$ more positive than the diet (Appendix 1). This is larger than the diet-

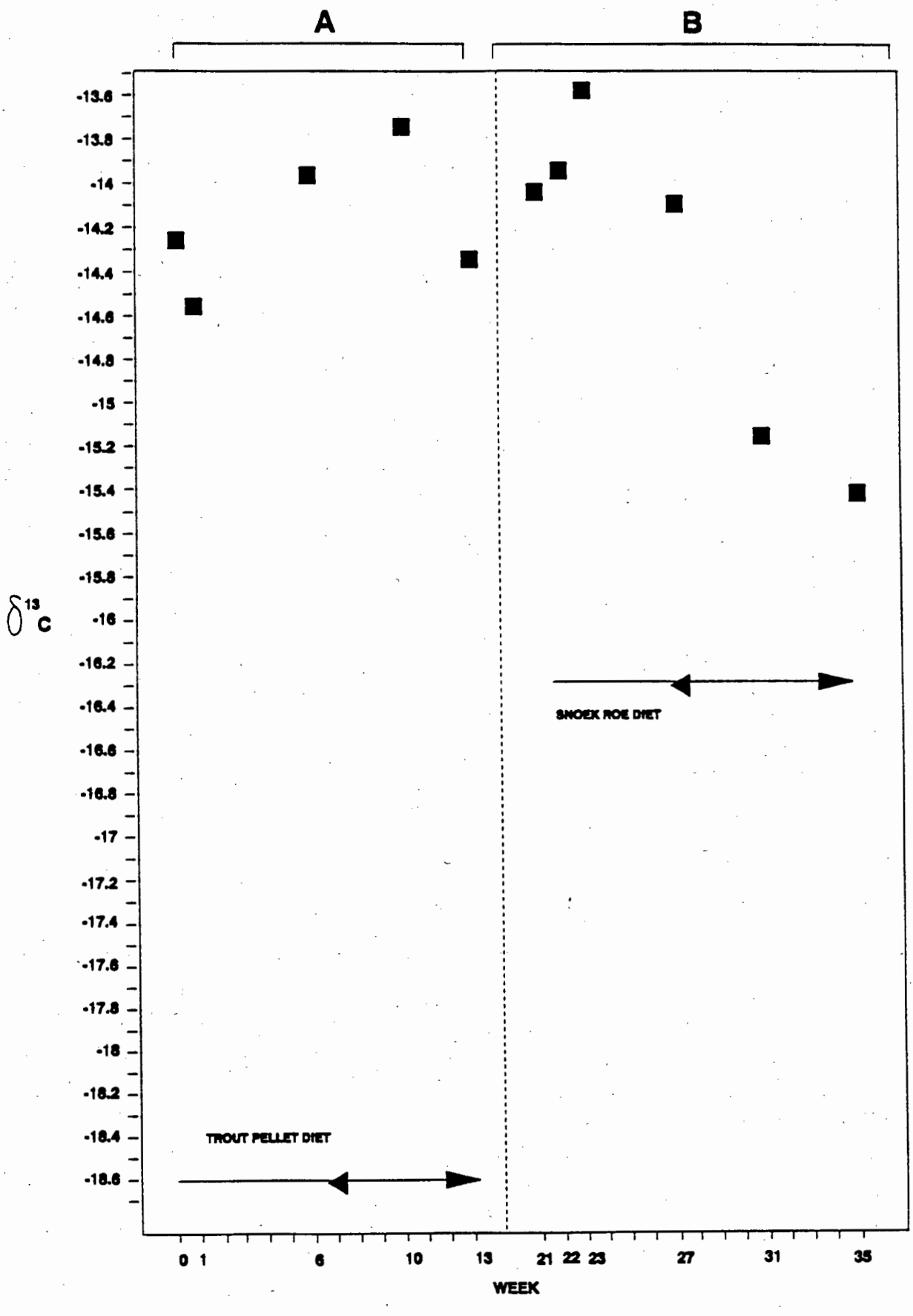


Fig. 2 $\delta^{13}C$ measurements for muscle tissue from adult sardine, Sardinops ocellatus, fed a diet of trout pellets (A) which was later changed to a diet of snoek roe (B). Each sample represents a different fish.

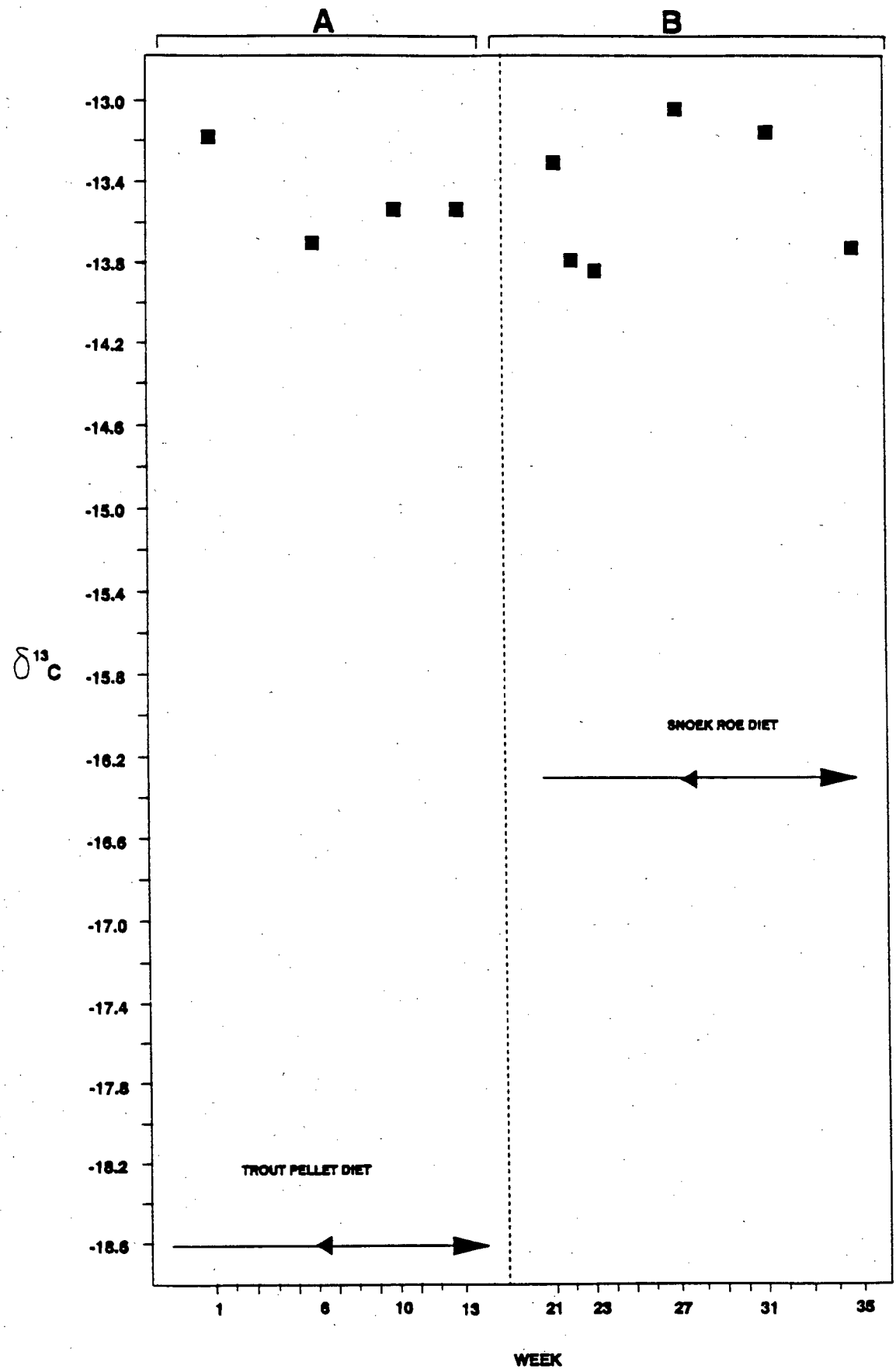


Fig. 3 $\delta^{13}C$ measurements for bone collagen tissue from adult sardine, Sardinops ocellatus, fed a diet of trout pellets (A) which was later changed to a diet of snoek roe (B). Each sample represents a different fish.

consumer muscle disparity reported in the literature for pelagic fish species (Sholto-Douglas et al. 1991). However, relative to the subsequent diet of snoek roe, this represents a $\delta^{13}C$ diet-consumer muscle enrichment of 2.0 $^{o}/_{oo}$, close to that reported in the literature. Thus no significant alteration in $\delta^{13}C$ was required for the muscle tissue to reflect their new diet. The decrease in $\delta^{13}C$ shown by the muscle tissue of sardine towards the end of the experiment, suggests that the diet-consumer disparity may be less than 2.0 $^{o}/_{oo}$ for $\delta^{13}C$, as has been found by Kling & Fry (1992) and Thayer et al. (1983) for pelagic organisms.

Fig. 3 shows the $\delta^{13}C$ results for bone collagen tissue. The mean $\delta^{13}C$ content of the bone collagen of fish fed trout pellets (-13.5 $^{o}/_{oo}$) was 5.0 $^{o}/_{oo}$ more positive than trout pellets (Appendix 1), slightly greater than the diet-consumer bone collagen $\delta^{13}C$ disparity reported by Sholto-Douglas et al. (1991), but of the same magnitude as that reported by Lee-Thorp et al. (1989) for terrestrial animals. Relative to the snoek roe however, the mean $\delta^{13}C$ content of the bone collagen of fish fed trout pellets was more positive, but by a smaller amount, 2.8 $^{o}/_{oo}$, not much less than the 3.2 $^{o}/_{oo}$ diet-consumer bone collagen disparity reported by Sholto-Douglas et al. (1991) for Cape anchovy (E. capensis). Hence bone collagen contained relatively more ^{13}C than muscle tissue. The $\delta^{13}C$ data for the sardine bone collagen show no response to the change in diet.

The $\delta^{15}N$ measurements for muscle tissue (Fig. 4) become more positive after the change to the new diet (R_s = 0.99, df = 5, $p < 0.05$). However it appears that the initial response slows down during weeks 27 to 35. The mean $\delta^{15}N$ content of the muscle from fish fed trout pellets was 12.6 $^{o}/_{oo}$, 1.2 $^{o}/_{oo}$ more positive than the $\delta^{15}N$ mean for trout pellets, but 2.8 $^{o}/_{oo}$ more negative than the snoek roe (Appendix 1). Allowing for a diet-consumer muscle enrichment of ca. 4 $^{o}/_{oo}$ for $\delta^{15}N$ (De Niro & Epstein 1978; McConnaughey & McRoy 1979; Minagawa & Wada 1984; Sholto-Douglas et al. 1991), a change of ca. 7 $^{o}/_{oo}$ was required for the fish to isotopically reflect the diet of snoek roe. At the end of the experiment the sardine muscle tissue was only 0.9 $^{o}/_{oo}$ more negative than the mean $\delta^{15}N$ value for snoek roe. The magnitude of change in $\delta^{15}N$ while the roe diet was administered was 1.9 $^{o}/_{oo}$ for muscle tissue. Taking into account that the fish were fed snoek roe for 14 weeks, this represents a daily rate of change in $\delta^{15}N$ of ca. 0.02 $^{o}/_{oo}$ (by proportion). Alternatively, the turnover rate (FSR) may be expressed as 0.6

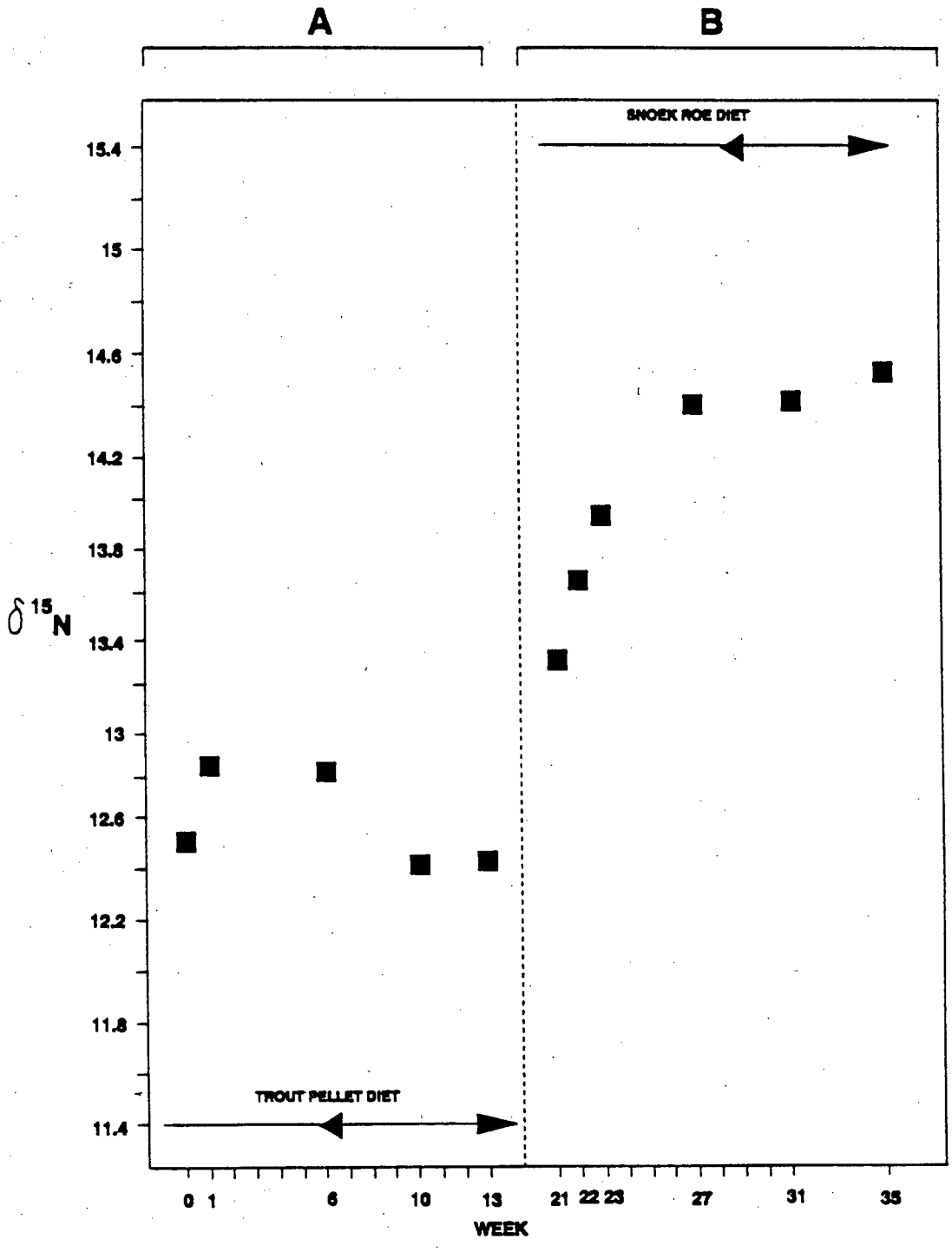


Fig. 4 $\delta^{15}N$ measurements for muscle tissue from adult sardine, Sardinops ocellatus, fed a diet of trout pellets (A) which was later changed to a diet of snoek roe (B). Each sample represents a different fish.

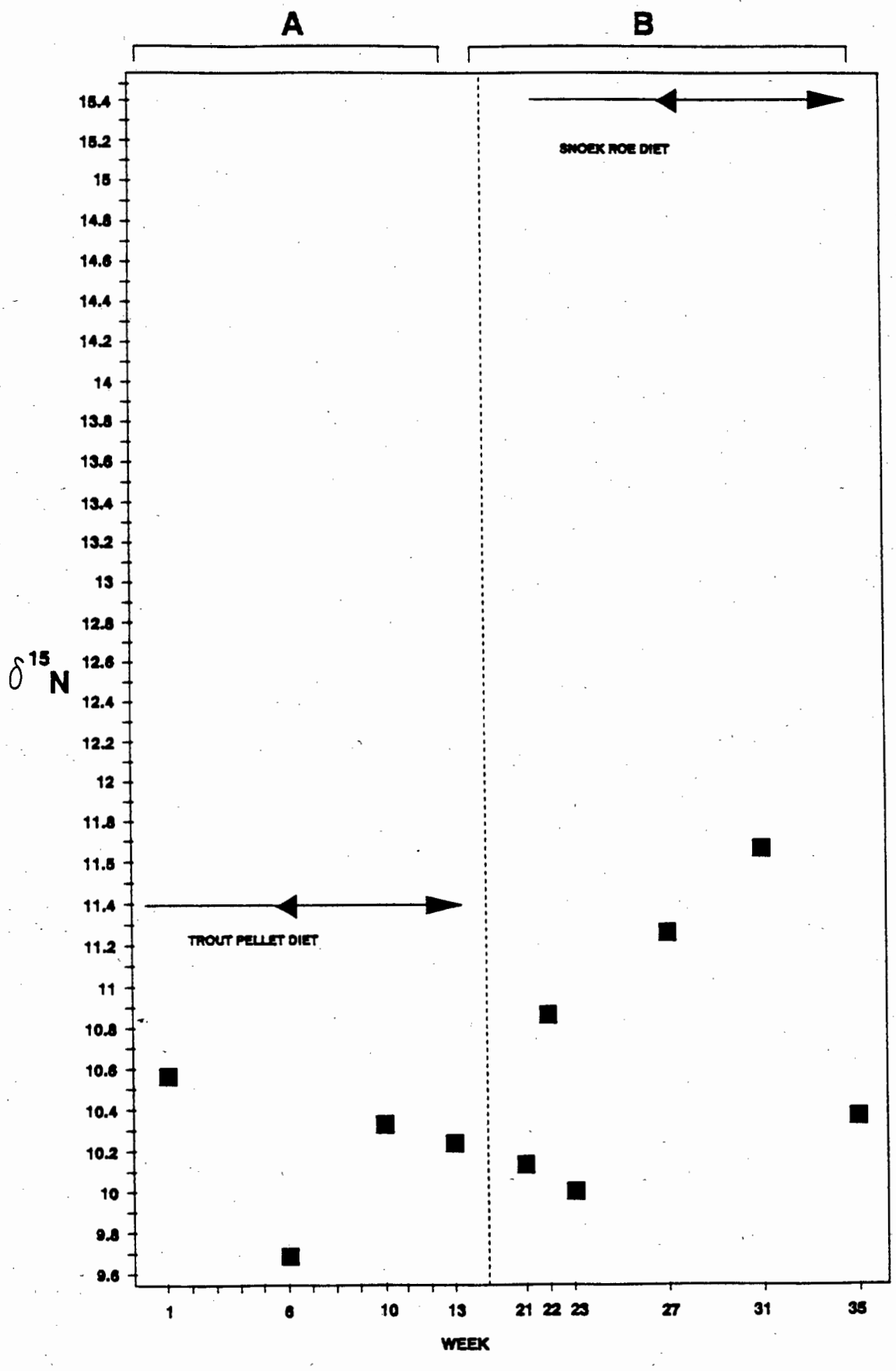


Fig. 5 $\delta^{15}N$ measurements for bone collagen tissue from adult sardine, Sardinops ocellatus, fed a diet of trout pellets (A) which was later changed to a diet of snoek roe (B). Each sample represents a different fish.

$^{o}/_{oo}$ on a monthly basis, or 7 $^{o}/_{oo}$ per year. Sardine muscle tissue would take nearly a year (10.3 months) to reflect the change in diet.

As for muscle tissue, the $\delta^{15}N$ measurements for bone collagen (Fig. 5) become more positive after the change to the new diet, but the change is less clear than for muscle tissue (R_S = 0.43, df = 5, $p > 0.05$) since some fish did not exhibit an increase in the $\delta^{15}N$ make-up of their bone collagen tissue. Nevertheless the average $\delta^{15}N$ make-up of the bone collagen tissue of fish fed trout pellets was ca. 10.2 $^{o}/_{oo}$, 1.2 $^{o}/_{oo}$ more negative than the diet, but 5.2 $^{o}/_{oo}$ more negative than the subsequent diet of snoek roe. Allowing for a diet-consumer bone collagen enrichment of ca. 2-2.5 $^{o}/_{oo}$ for $\delta^{15}N$ (Sholto-Douglas et al. 1991) a change of 7.2-7.7 $^{o}/_{oo}$ was required for the fish to isotopically reflect a diet of snoek roe. Not all the bone collagen samples showed a response to the new diet. The best estimate of the change in bone collagen $\delta^{15}N$ due to the change in diet is the difference between the $\delta^{15}N$ means for fish fed trout pellets and those fed snoek roe (10.2 $^{o}/_{oo}$ and 10.7 $^{o}/_{oo}$), which is 0.5 $^{o}/_{oo}$. Taking into account that the fish were fed snoek roe for 14 weeks, this represents a daily rate of change in $\delta^{15}N$ of 0.005 $^{o}/_{oo}$. Alternatively, the turnover rate (FSR) may be expressed as 0.15 $^{o}/_{oo}$ per month and 1.9 $^{o}/_{oo}$ per year. A total change in $\delta^{15}N$ of ca. 7 $^{o}/_{oo}$ is required for the sardine bone collagen to isotopically reflect the diet of snoek roe, assuming a 2-2.5 $^{o}/_{oo}$ enrichment from diet to bone collagen tissue (Sholto-Douglas et al. 1991). Hence, sardine muscle tissue would take about 3.5 years to reflect the change in diet, nearly 4 times the time required for muscle tissue.

Bone collagen had more negative $\delta^{15}N$ values than muscle tissue throughout the experiment. The average difference was 2.6 $^{o}/_{oo}$ while the fish were fed trout pellets but 3.3 $^{o}/_{oo}$ during the time they were fed snoek roe. The $\delta^{15}N$ disparity between these tissues for sardine was within the range reported by Sholto-Douglas et al. (1991) for pelagic fish from southern African waters (mean differences of 4.3 $^{o}/_{oo}$ and 2.4 $^{o}/_{oo}$ for E. capensis and E. whiteheadi respectively).

During the 13 weeks the isotopic make-up of the sardine consuming trout pellets was monitored, they appeared to be close to a state of isotopic equilibrium with their food, since no change in the isotopic make-up of their tissues is apparent. However, it is interesting that the fish feeding on trout pellets do not exhibit normal consumer-diet

Table 3

Diet Consumed		Hindgut contents	Fish muscle	Bone collagen
Plankton	-17.3 ± 1.1 (n=6)	-15.8 ± 0.2 (n=7)	-15.8 ± 0.2 (n=7)	-14.5 (n=3)
Pellets	-18.6 ± 0.03 (n=3)	-15.6 ± 0.3 (n=5)	-14.3 ± 0.2 (n=5)	-13.4 ± 0.1 (n=4)
Roe	-16.3 ± 0.1 (n=4)	-17.5 ± 0.3 (n=2)	-14.4 ± 0.3 (n=6)	-13.4 ± 0.1 (n=6)

Table 3 Mean $\delta^{13}C$ values for <200 μm plankton from the Agulhas Bank, trout pellets and snoek roe versus the average isotopic make-up of the tissues of sardine (Sardinops ocellatus) utilising these food sources. Standard errors are given, as are numbers of samples (n). The standard errors for the bone collagen tissue of sardine from the Agulhas Bank are not presented, since the isotope value was estimated from the results for muscle tissue in keeping with the muscle-bone collagen relationships reported by Sholto-Douglas et al. (1991).

Table 4

Diet Consumed		Hindgut contents	Fish muscle	Bone collagen
Plankton	7.5 ± 0.2 (n=4)	9.0 ± 0.2 (n=7)	11.2 ± 0.1 (n=7)	8.9 (n=3)
Pellets	11.4 ± 0.06 (n=3)	13.2 ± 0.1 (n=5)	13.5 ± 0.2 (n=5)	10.2 ± 0.2 (n=4)
Roe	15.4 ± 0.2 (n=5)	15.0 ± 0.7 (n=2)	14.5 ± 0.2 (n=6)	10.7 ± 0.3 (n=6)

Table 4 Mean $\delta^{15}N$ values for <200 μm plankton from the Agulhas Bank, trout pellets and snoek roe versus the average isotopic make-up of the tissues of sardine (Sardinops ocellatus) utilising these food sources. Standard errors are given, as are numbers of samples (n). The standard errors for the bone collagen tissue of sardine from the Agulhas Bank are not presented, since the isotope value was estimated from the results for muscle tissue in keeping with the muscle-bone collagen relationships reported by Sholto-Douglas et al. (1991).

they had been fed trout pellets for over a year. The dietary sequence from plankton to trout pellets to snoek roe represents a 4 $^{o}/_{oo}$ increase in $\delta^{15}N$ with each dietary change. The $\delta^{15}N$ values for sardine muscle tissue become more positive in response to the increase in the $\delta^{15}N$ content of their respective diet, but by smaller amounts than is evident for their hindgut contents, suggesting a delay associated with the period of isotopic integration into the fish tissues (Table 4). These results are in keeping with the findings of Checkley & Entzeroth (1985) who found that the isotopic make-up of copepod bodies and faeces was more positive than that of their potential diet.

The $\delta^{13}C$ and $\delta^{15}N$ values for the hindgut contents of fish caught in their natural environment from the region of the Agulhas Bank are more positive than those for their potential diet (<200 μm plankton) by 1.5 $^{o}/_{oo}$ in both cases (Tables 3 and 4). The hindgut contents of the fish fed trout pellets are more enriched in ^{13}C and ^{15}N than their corresponding food source, as is the case for fish from the Agulhas Bank. They have similar $\delta^{13}C$ values to the hindgut contents of Agulhas Bank sardine in spite of a slight increase in the mean $\delta^{13}C$ content of their food, suggesting that their dietary carbon was adequate and excess ^{13}C was dispensed with by respiration, or in non-particulate waste matter. For $\delta^{15}N$ however, their hindgut contents are more positive than the hindgut contents of fish from the Agulhas Bank, by ca. 4 $^{o}/_{oo}$, corresponding to the $\delta^{15}N$ increase in their diet (Table 4). The hindgut contents of the sardine fed snoek roe are not isotopically more positive than their diet, since they become enriched in ^{15}N by half the corresponding increase in their diet (Table 4), while they become depleted in ^{13}C in contrast to the trend of ^{13}C enrichment in their diet (Table 3). It appears that they were further from a state of isotopic equilibrium than the fish fed trout pellets, and more ^{15}N was being assimilated by the tissues than excreted to acquire the $\delta^{15}N$ content in their tissues associated with that in their food.

Assuming that the isotopic make-up of the sardine used in the experiment was originally similar to those of the sardine from the Agulhas Bank, the total magnitude of change in their muscle tissue was 1.5 $^{o}/_{oo}$ for $\delta^{13}C$ and 2.3 $^{o}/_{oo}$ for $\delta^{15}N$ in 17 months (14+3 because the fish were fed trout pellets for 3 months after the experiment began). Assuming that the $\delta^{13}C$ content of bone collagen is more positive than that of muscle tissue by ca. 1.3 $^{o}/_{oo}$, but for $\delta^{15}N$, more negative by ca. 2.3 $^{o}/_{oo}$ (Sholto-Douglas et al. 1991), the change in bone collagen during this time was ca. 1.1 $^{o}/_{oo}$ for $\delta^{13}C$ and 1.3 $^{o}/_{oo}$

for $\delta^{15}N$. This represents a $\delta^{13}C$ turnover rate of ca. 0.1 $^{o}/_{oo}$ month^{-1} (1 $^{o}/_{oo}$ year^{-1}) for both sardine muscle and bone collagen tissues. The $\delta^{15}N$ turnover rate for sardine bone collagen is also ca. 0.1 $^{o}/_{oo}$ month^{-1} (1 $^{o}/_{oo}$ year^{-1}), but for sardine muscle tissue the $\delta^{15}N$ turnover is ca. 0.15 $^{o}/_{oo}$ month^{-1} (1.8 $^{o}/_{oo}$ year^{-1}), almost double. These are slower rates of turnover than calculated for the fish fed snoek roe. It is possible that the relative change in the isotopic ratio of the fish fed trout pellets decreased as the fish approached isotopic equilibrium with the food source (hence no noticeable isotopic change in the tissues of the fish fed trout pellets). The sardine in this experiment, did not easily consume trout pellets and often needed to be coaxed to feed. Their feeding behaviour was unenthusiastic and much of the food sank to the bottom of the pool where it was left untouched. Snoek roe, however, was ravished by the fish and little food would reach the bottom of the pool. Shoaling behaviour was initiated as soon as I appeared at the pool at feeding time, indicating expectancy and desire for the food. It is also possible that the apparently slow isotopic response in the fish tissues to their trout pellet diet was related to their dislike for the diet.

Checkley & Entzeroth (1985) found that the magnitude of the fractionation between the suspended particulate matter and faeces was greater for $\delta^{15}N$ (ca. +8 $^{o}/_{oo}$) than $\delta^{13}C$ (ca. +1 $^{o}/_{oo}$). The analogous fractionations for the body tissues were ca. +6 $^{o}/_{oo}$ and +2 $^{o}/_{oo}$. The $\delta^{15}N$ disparity between the hindgut contents of the sardine in this study are smaller. This may be because both soluble excreta and solid particulate faecal material were included in the analysis of hindgut contents. Checkley & Entzeroth (1985) found ammonium and respired CO_2 to be depleted in ^{15}N and ^{13}C, respectively.

SUMMARY

Throughout the experiment the $\delta^{13}C$ content of bone collagen tissue was more positive than that of muscle tissue, while the $\delta^{15}N$ content of muscle tissue was more positive than for bone collagen, as is the case for other pelagic fish species from southern African waters (Sholto-Douglas et al. 1991). Muscle tissue showed a clearer $\delta^{15}N$ response than bone collagen, probably because the turnover rate of muscle tissue is faster. Nevertheless, it is unlikely that short term dietary changes would influence the average isotopic make-up reflecting the "preferred" diet of these fish.

CHAPTER 4

FRACTIONATION WITH FISH LENGTH

INTRODUCTION

In pelagic ecosystems, the tissues of larger and longer lived organisms, such as fish, will isotopically reflect a diet integrated over a longer period than the tissue of smaller, shorter lived plankton species. Thus short term dietary changes may not easily be distinguished on the basis of the isotopic make-up of their tissues (chapter 3). However, the bulk diet of a fish may change during its lifetime (King & Macleod 1976) so that their average juvenile diet may be isotopically distinct from their adult diet, which may be reflected in their tissues.

Sampling

Fig. 6 shows the positions in which the fish were caught, as well as plankton samples, the results of which are compared to those for the fish tissues in chapter 5.

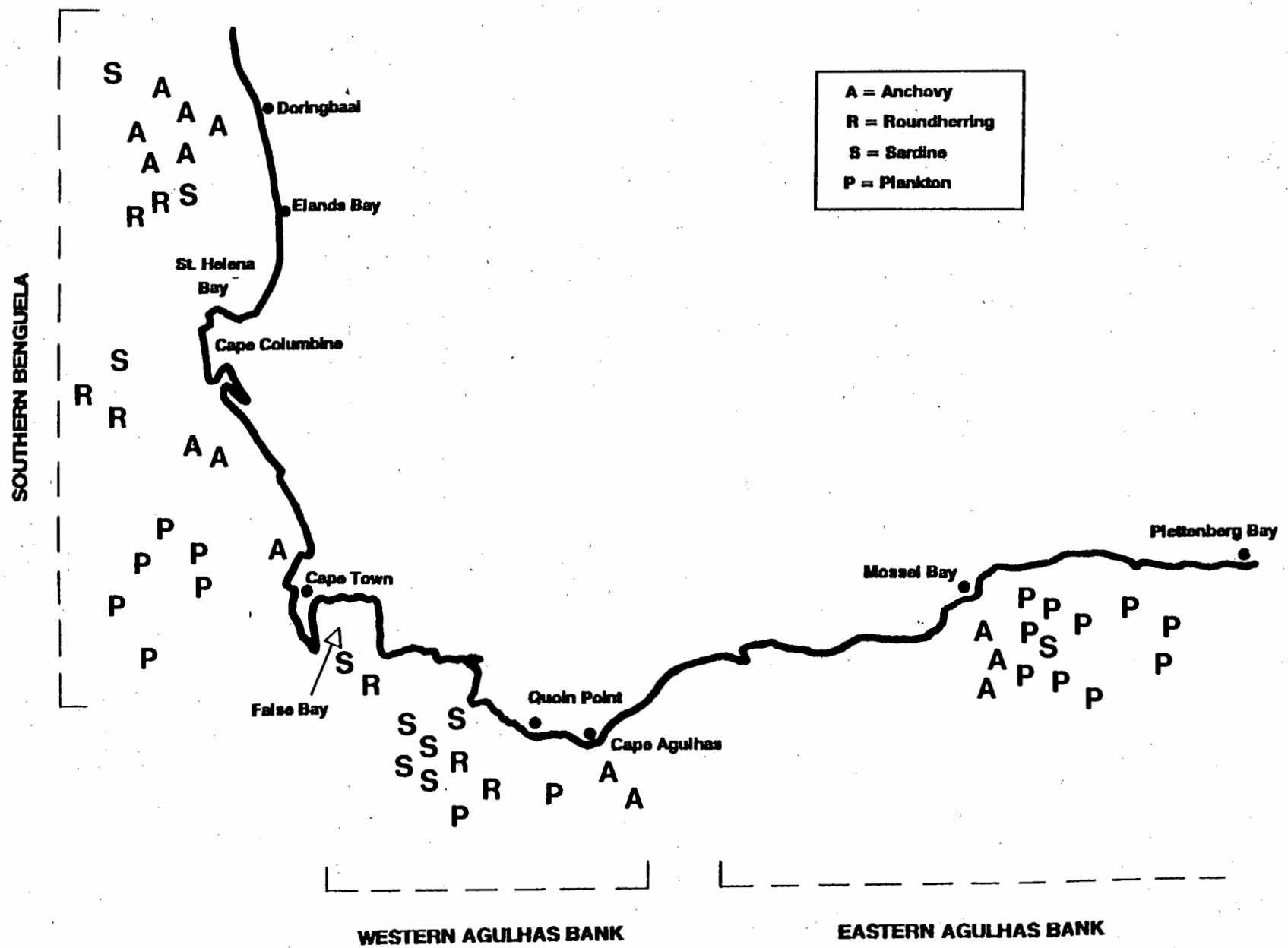
Southern Benguela ecosystem

Samples of anchovy, Engraulis capensis, and round-herring, Etrumeus whiteheadi, were collected off the west coast of South Africa, in the region between Doringbaai and Ysterfontein (see Fig. 6), during cruises aboard R.S. Africana in May 1986 and July 1987 using an Engels 308 midwater trawl. Sardine, Sardinops ocellatus samples were collected from the same area in July 1987, but none were obtained in May 1986.

Agulhas Bank

In November 1989, samples of anchovy and sardine were obtained south and east of Cape Point during a cruise aboard the RV Africana. Sardine were caught west of Cape Agulhas (except one), in the region between Cape Point and Quoin Point (Fig. 6). This region may be described as the western Agulhas Bank (Shannon & Pillar 1986), but is usually included as part of the southern Benguela system. Anchovy were caught east of Cape

Fig. 6 Map showing the plankton and fish sampling areas.



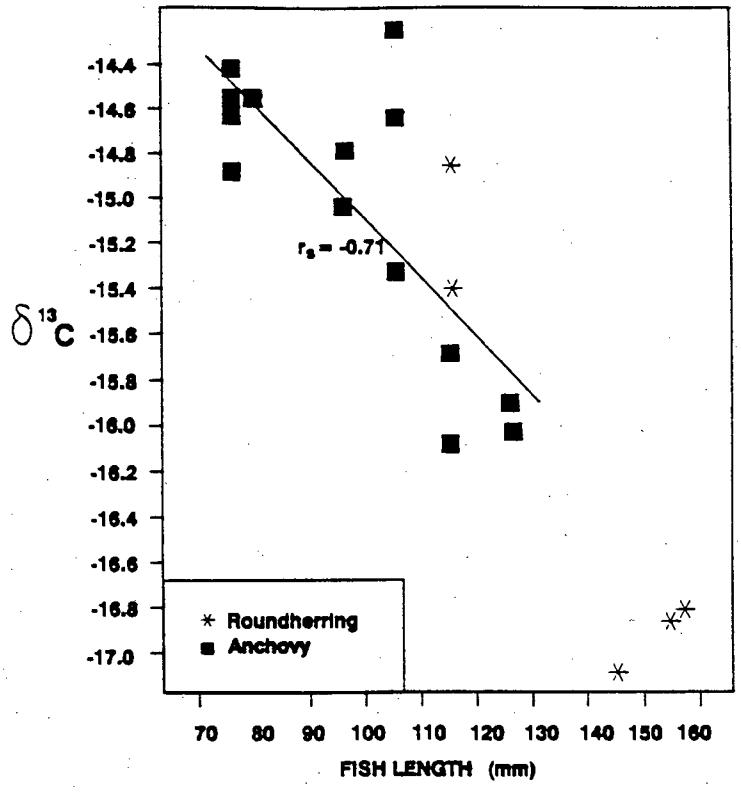


Fig. 7 $\delta^{13}C$ measurements for muscle tissue from different size-classes of anchovy, Engraulis capensis, and roundherring, Etrumeus whiteheadi, from the west coast of South Africa (southern Benguela ecosystem). Regression lines are given for anchovy, as are Spearman's rank-correlation coefficients (r_S).

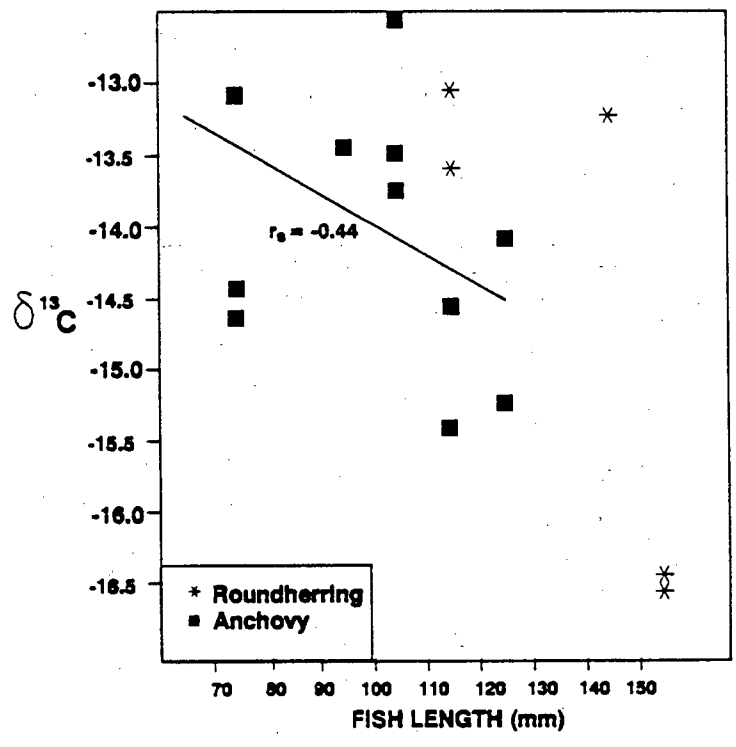


Fig. 8 $\delta^{13}C$ measurements for bone collagen tissue from different size-classes of anchovy, Engraulis capensis, and roundherring, Etrumeus whiteheadi, from the west coast of South Africa. Regression lines are given for anchovy, as are Spearman's rank-correlation coefficients (r_S).

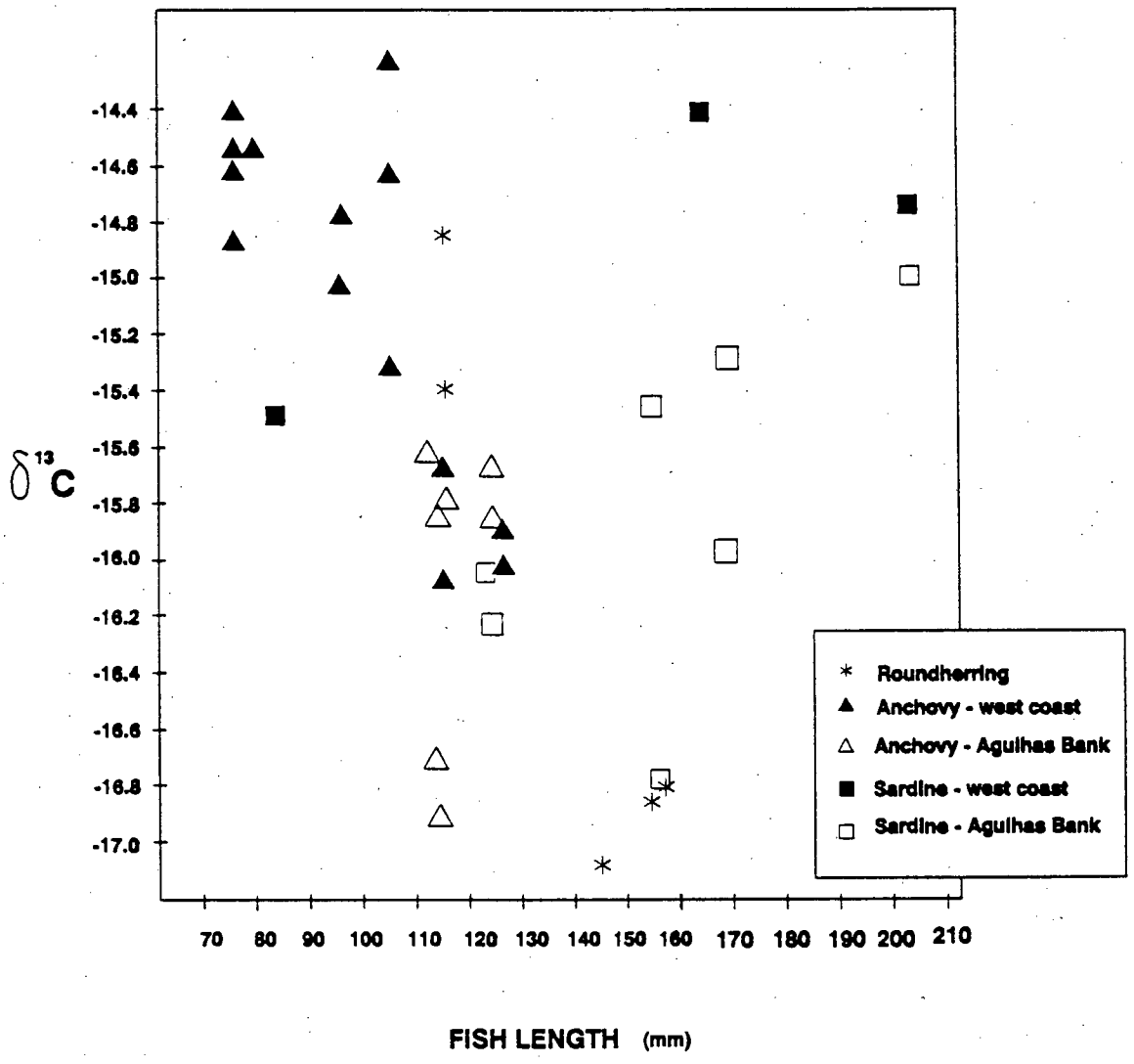


Fig. 9 $\delta^{13}C$ measurements for the muscle tissue of different size-classes of anchovy, roundherring and sardine, Engraulis capensis, Etrumeus whiteheadi and Sardinops ocellatus, from the west coast of South Africa as well as anchovy and sardine from the Agulhas Bank area.

collagen tends to be more positive than muscle tissue. As for muscle tissue, the $\delta^{13}C$ values for anchovy bone collagen tend to become more negative with increasing fish size, but the decrease is not statistically significant (R_S = -0.47, df = 12, $p > 0.05$). Sample sizes for roundherring are small for correlations, but a similar trend of more negative $\delta^{13}C$ values with increasing fish length appears to exist for both muscle and bone collagen tissues.

Fig. 9 shows the $\delta^{13}C$ values for the muscle tissue of all species of fish (including sardine) from both sampling areas. Bone collagen tissue was not measured for fish from the Agulhas Bank. The anchovy caught from the Agulhas Bank are all 110-130mm caudal length, representing fish >1 year old year old that have probably recently recruited from the west coast to the spawning area (Waldron et. al. 1989). The $\delta^{13}C$ content of their muscle tissue is more negative than the muscle tissue of fish less than 110mm caudal length, as was the case for anchovy of this size from the west coast. The muscle tissue of anchovy from both areas together shows a stronger negative correlation between the $\delta^{13}C$ and fish length (R_S = -0.81, df = 9, $p < 0.05$) than exists for the west coast data alone.

The $\delta^{13}C$ values for the muscle tissue of sardine caught from the Agulhas Bank tend to be more negative than those for sardine from the west coast, but there is no significant difference between the data for sardine muscle from the two areas. The $\delta^{13}C$ values for sardine appear to become more positive with increasing fish length (R_S = 0.75, df = 6, $p < 0.1$ for the Agulhas Bank data, R_S = 0.67, df = 9, $p < 0.05$ for sardine from both areas together).

Fig. 10 depicts the relationship between fish size and the $\delta^{15}N$ ratios of the muscle tissue from anchovy and roundherring caught from the southern Benguela ecosystem. The $\delta^{15}N$ values for anchovy muscle tissue range from 12.3 to 13.6 $^{o}/_{oo}$, while roundherring values lie between 12.8 and 15.1 $^{o}/_{oo}$. There is little sign of a $\delta^{15}N$ relationship with fish length (R_S = -0.08, df = 14, $p > 0.05$) for anchovy.

The $\delta^{15}N$ values for anchovy bone collagen tissue range from 9.8 to 11.8 $^{o}/_{oo}$ and between 10.1 and 13.2 $^{o}/_{oo}$ for roundherring (Fig. 11). Therefore in contrast to $\delta^{13}C$, the muscle tissues of these fish species tend have more positive $\delta^{15}N$ values than bone collagen tissue. Furthermore, the $\delta^{15}N$ values for anchovy bone collagen show a stronger negative

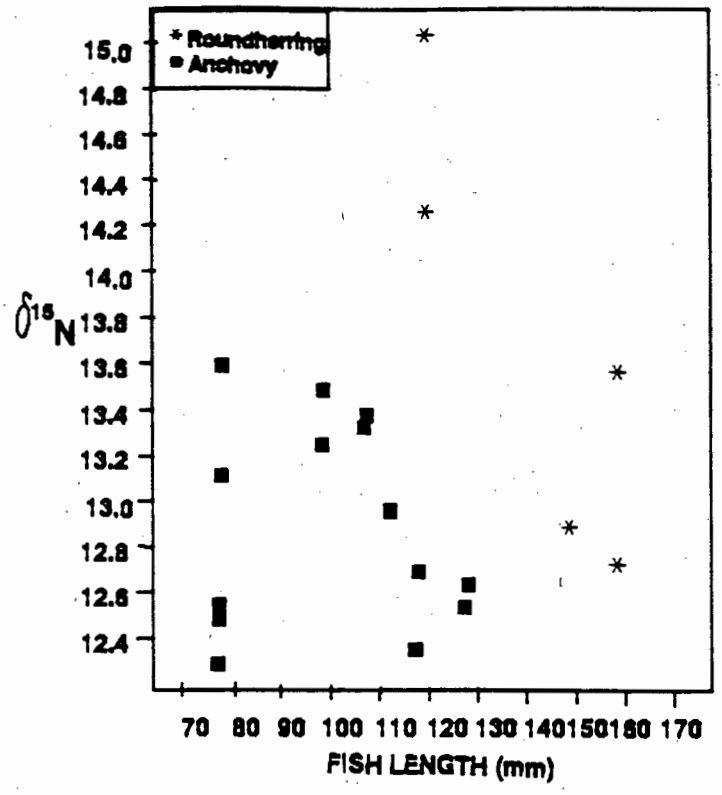


Fig. 10 $\delta^{15}N$ measurements for the muscle tissue of different size-classes of anchovy, Engraulis capensis, and roundherring, Etrumeus whiteheadi, from the west coast of South Africa.

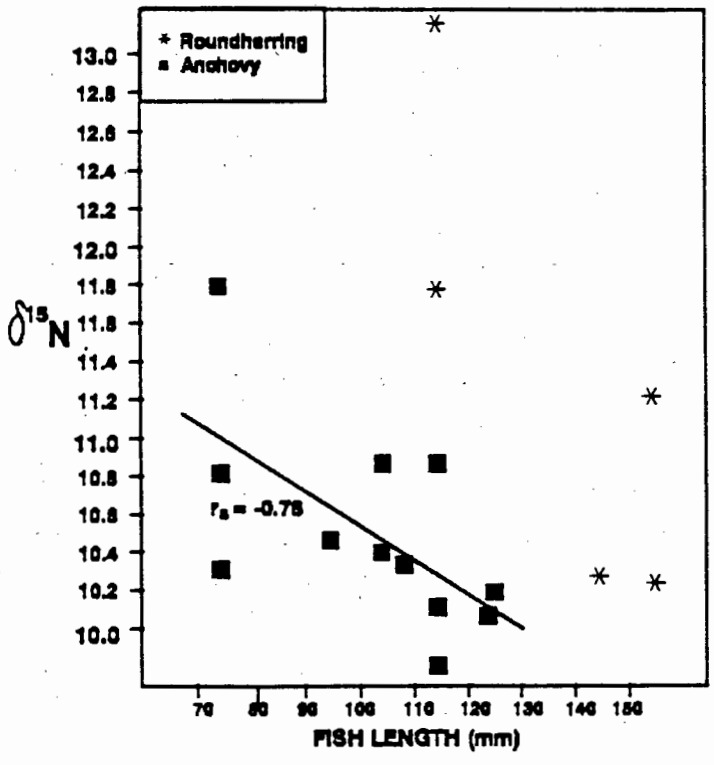


Fig. 11 $\delta^{15}N$ measurements for the bone collagen tissue of different size-classes of anchovy, Engraulis capensis, and roundherring, Etrumeus whiteheadi, from the west coast of South Africa. Regression lines are given for anchovy, as are Spearman's rank-correlation coefficients (r_S).

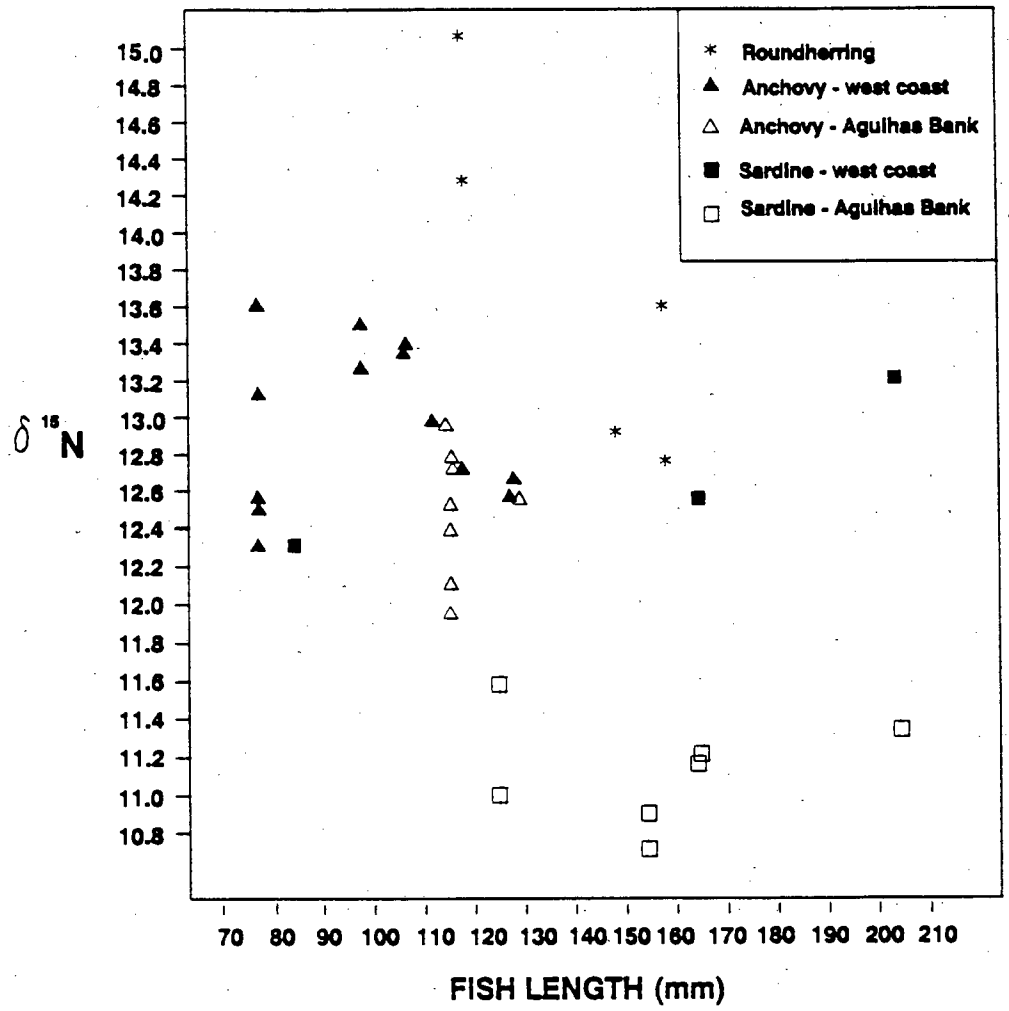


Fig. 12 $\delta^{15}N$ measurements for the muscle tissue of different size-classes of anchovy, roundherring and sardine, Engraulis capensis, Etrumeus whiteheadi and Sardinops ocellatus, from the west coast of South Africa as well as anchovy and sardine from the Agulhas Bank area.

correlation with increasing fish length (R_s = -0.71, df=12, $p<0.02$) than muscle. It is interesting to note that the tissue with more negative $\delta^{13}C$ or $\delta^{15}N$ values appears to show a stronger relationship with fish length. The isotopic variability of anchovy in the 70-80 mm size-class may be explained by the possibility that some of these fish still reflect the integrated isotopic signature of a different juvenile diet. The omission of these values would result in good negative correlations with increasing anchovy length in both their muscle and bone tissues, for $\delta^{15}N$ and $\delta^{13}C$.

There appears to be a trend whereby the $\delta^{15}N$ make-up of roundherring tissues become more negative with increasing fish size.

Fig. 12 shows the $\delta^{15}N$ values for the muscle tissue of all three species of fish, from both sampling areas, versus organism size. As was the case for anchovy from the west coast, the muscle tissue of the larger anchovy caught from the Agulhas Bank have more negative $\delta^{15}N$ values than the muscle tissue of anchovy less than 110mm caudal length, resulting in a stronger negative relationship with fish length for muscle tissue (R_s = -0.34, df = 20, $p<0.1$) $\delta^{15}N$.

The $\delta^{15}N$ content of the muscle tissue of sardine from the Agulhas Bank are more negative than the muscle tissue of sardine from the west coast, as was the case for $\delta^{13}C$, but for $\delta^{15}N$ the difference is significant (U = 21; $p<0.01$). Thus it is possible that sardine from the Agulhas Bank consume a diet that is isotopically more negative than those from the west coast, but it is difficult to draw any conclusive comparisons due to the small sample size for west coast sardine. The muscle tissue of sardine from the Agulhas Bank also have more negative $\delta^{15}N$ values than the other fish species, suggesting that this is the case for their diet. No relationship with sardine length is evident in the $\delta^{15}N$ data (R_s = 0.27, df = 9, $p>0.05$).

Figs. 13 a and b show the results for the gut contents of anchovy caught from the southern Benguela ecosystem. Figs. 14 a and b show the results for the gut contents of roundherring from the same area. No relationship with fish length was exhibited by the results for gut contents. The gut contents at the time the fish were caught may not necessarily be representative of their average dietary history. If the isotopic relationships with increasing fish length have a trophic basis, and larger fish are consuming greater

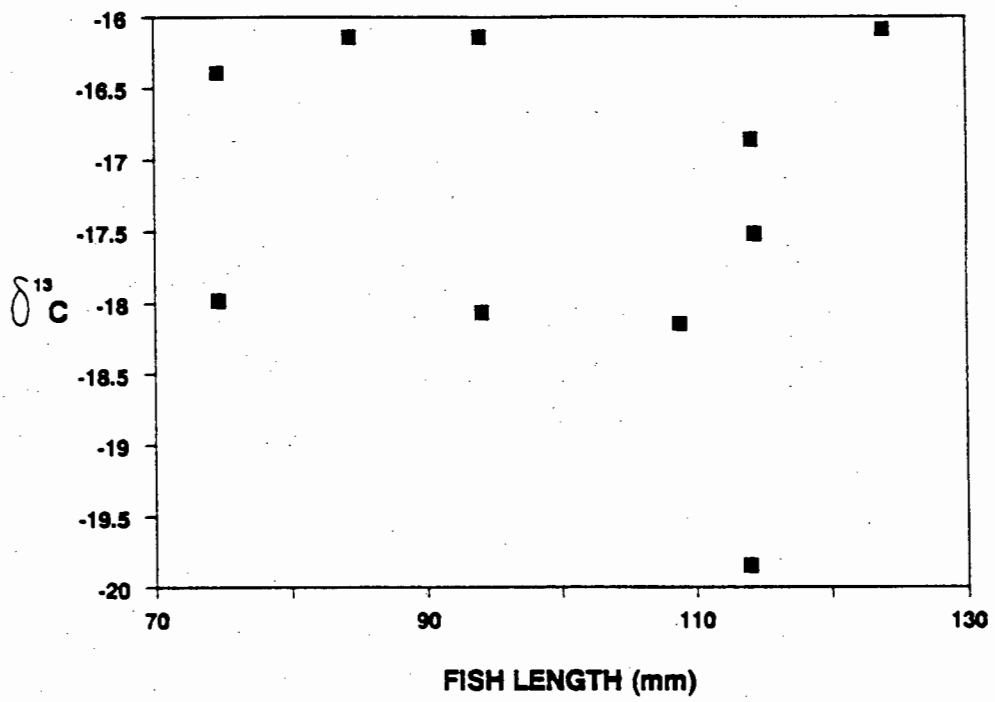


Fig. 13 a $\delta^{13}C$ values for the gut contents of different size-classes of anchovy caught from the west coast of South Africa

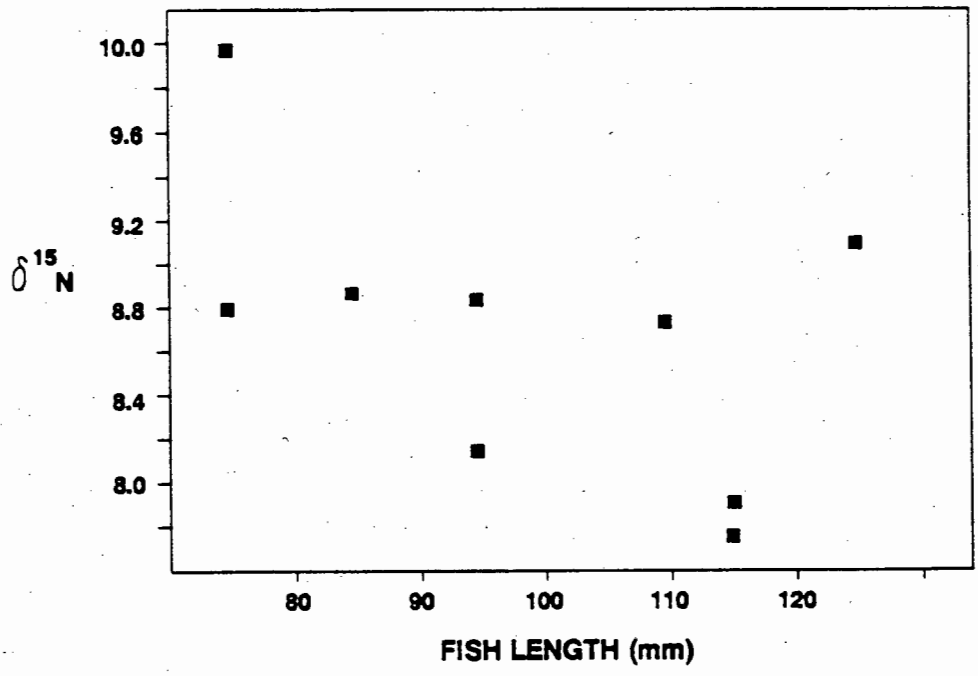


Fig. 13 b $\delta^{15}N$ values for the gut contents of different size-classes of anchovy caught from the west coast of South Africa

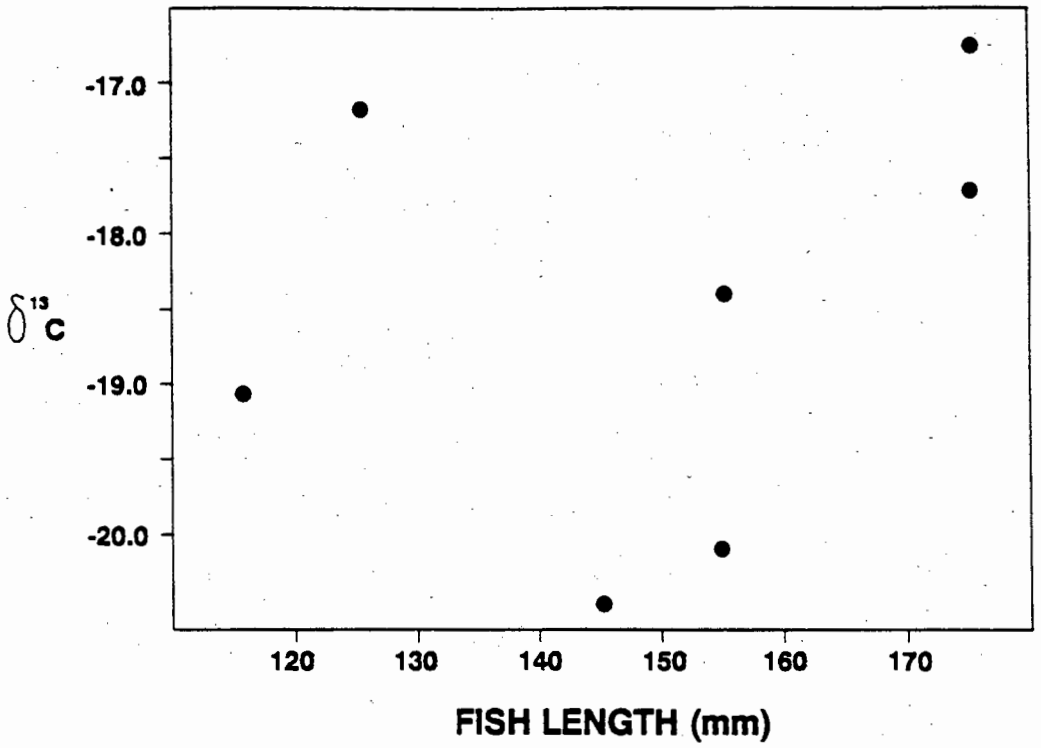


Fig. 14 a $\delta^{13}C$ values for the gut contents of different size-classes of roundherring caught from the west coast of South Africa

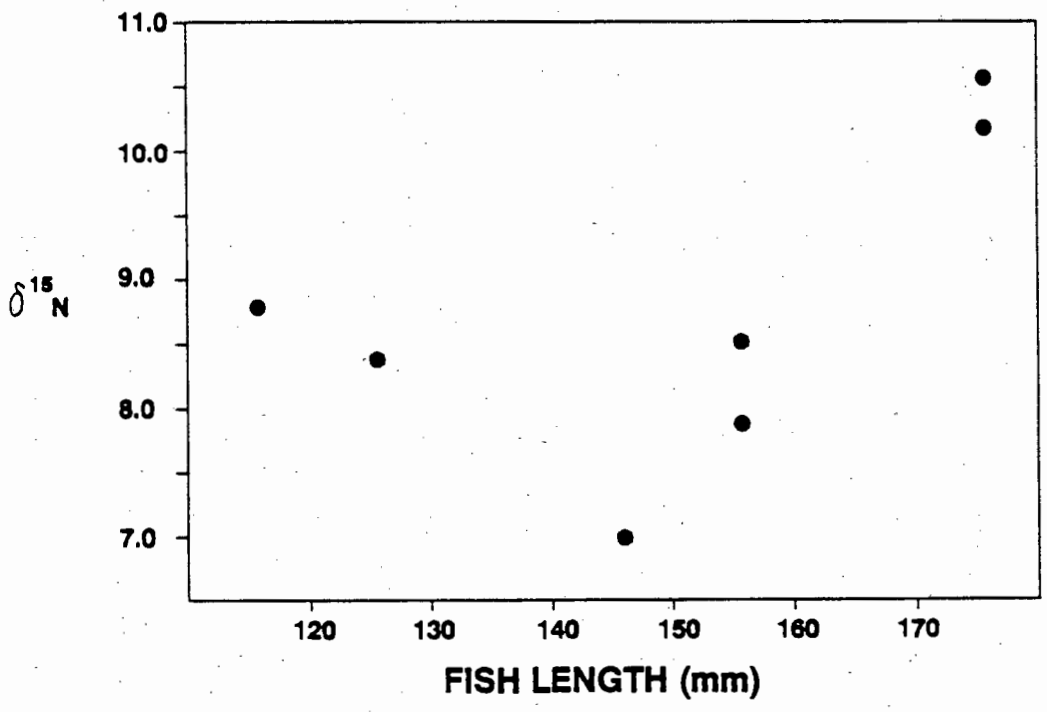


Fig. 14 b $\delta^{15}N$ values for the gut contents of different size-classes of roundherring caught from the west coast of South Africa

proportions of a relatively isotopically negative diet such as phytoplankton, then the results, to some extent, support the findings of King & Macleod (1976), who found that anchovy switch their diet at about the 80 mm size-class, from a predominance of zooplankton to a predominance of phytoplankton. If however, the different size-classes of fish had been consuming similar average diets, as indicated by their gut contents, then the relationships with fish length probably have a physiological basis. We have found no reference to differential incorporation of ^{13}C and ^{15}N by muscle and bone tissues. Nevertheless, there is reference to $\delta^{15}N$ relationships with body size. Rau et al. (1981) found a strong positive correlation between the $\delta^{15}N$ values of muscle tissue from Dover Sole with increasing body weight. However, Minagawa & Wada (1984) found no $\delta^{15}N$ correlation with age in mussels. Differences in Growth Efficiencies, or Assimilation Efficiencies, as described by Owens (1987), may be responsible for ^{13}C and ^{15}N variability with age/body size, but we cannot explain the different directions of the relationships found by these authors and ourselves.

SUMMARY

The $\delta^{13}C$ and $\delta^{15}N$ values for the muscle and bone collagen tissues from both anchovy and roundherring from the west coast become more negative with increasing fish length. The addition of the isotopic data for >110 mm (caudal length) fish from the Agulhas Bank to those for fish from the west coast results in stronger negative correlations with fish length for both $\delta^{13}C$ and $\delta^{15}N$.

Bone collagen tends to have more positive $\delta^{13}C$ values than muscle tissue, but muscle tissue has more positive $\delta^{15}N$ values than bone collagen. These relationships require further study both in the field and laboratory.

CHAPTER 5

FOOD WEB STRUCTURE

INTRODUCTION

Each oceanic water mass has its own species distribution, seasonal variation and productivity governing variations of isotopic composition. Thus water masses are isotopically distinct from one another (Fontugne & Duplessy 1978) and so too are the isotopic compositions of the organisms within them. Hence it is important to get an idea of the isotopic composition of plankton from both the southern Benguela and Agulhas Bank regions off southern Africa, since pelagic fish species are dependant upon plankton in these two regions (see section 1.4.3). Furthermore, the isotopic make-up of plankton may vary considerably, depending on the number of trophic interactions within the foodweb and other factors (chapter 1). Larger plankton are able to consume a greater size-range of plankton smaller than themselves. Thus it is useful to compare the isotope values obtained for the fish gut contents and tissues with those of different size-classes of plankton caught in the water at a similar time of year, and assess the isotopic value of the potential available food source in the water column.

Plankton Sampling

- Southern Benguela ecosystem

Plankton was collected from the west coast of Africa in July 1987, but samples were too small for isotope analyses. We therefore re-sampled in the same area in late May and June 1988 (see Fig. 6). Samples were collected with a multiple opening/closing rectangular midwater trawl (RMT 1x6) and sorted into 20-200 μm, 200-500 μm and >500 μm escape diameter size-classes, by washing fresh samples through screens aboard ship with seawater. The three size-classes comprised mainly phytoplankton and microzooplankton, copepods, and euphausiids respectively.

- Agulhas Bank

Plankton from the Agulhas Bank was collected during a cruise aboard the R.V. Meiring Naude in November 1989. Most of the samples were obtained offshore of Mossel Bay, east of the region fish were caught (the spawning area, see Fig. 6). Sampling was carried out with vertical hauls using a BONGO net (200 μm mesh diameter) and oblique tows using a 300 μm mesh diameter neuston net. Sampling depth did not exceed 50m. These samples were screened on board into five size classes, viz. <200 μm, 200-500 μm, 500-1600 μm, 1600-3500 μm and >3500 μm. The >3500 μm size class comprised mainly fish and crab larvae (only one sample contained euphausiids), inappropriate for comparative analyses. Most of the plankton fell into the 200-500 μm and 500-1600 μm size fractions.

Samples were defatted as described in chapter 2, and stable isotope analyses carried out. Mann-Whitney U tests were performed, and their significance levels calculated, to compare the differences between the means of different size-classes of plankton, between fish gut contents and the different plankton size-classes, and between the muscle and bone collagen tissues of each species of fish. Kruskal-Wallis one-way ANOVA and nonparametric Tukey-type multiple comparison tests were carried out to determine differences between pooled values of gut contents, muscle and bone collagen tissues from the three species of fish from the west coast. Statistics were calculated according to Zar (1984).

Data from Shannon & Agenbag (1990) show similar mean Sea Surface Temperature (SST) anomalies in the southern Benguela region during July 1987 (-1^{o}C to 1^{o}C), July 1988 (mostly -1^{o}C to 0^{o}C) and May 1986 (1-2^{o}C). Using SST as an index of the environment, one can assume that conditions for plankton caught from the west coast during these months, were similar. However, the isotopic content of these organisms at the time of capture is not necessarily related to the environmental conditions at the same time. Conditions during the preceding months are also important. The first halves of 1986, 1987 and 1988 show slightly decreasing average anomalies from +1^{o}C to -1^{o}C (Shannon & Agenbag 1990). This interannual variation is small and unlikely to cause temporal bias in the data for organisms from the west coast.

RESULTS AND DISCUSSION

5.1 WITHIN THE PLANKTON

The $\delta^{13}C$ data obtained for the different size classes of plankton caught from the west coast are shown in Fig. 15. The $\delta^{13}C$ values of larger plankton tend to become more positive with increasing plankton size, suggesting that larger plankton feed further up the foodweb than do smaller plankton. There is much scatter in the data, which decreases with increasing size class. This suggests that primary producers, primary and secondary consumers, may all be represented in the 20-200 μm size fraction. Thus the values for each size-class are represented within the ranges of the smaller size-classes. The differences between the means of the $\delta^{13}C$ data for successive size classes are not significant (Table 5).

Fig. 16 shows the $\delta^{13}C$ data obtained for plankton caught from the Agulhas Bank region in November 1989 (statistics in Table 5). It is important to be aware that these plankton were separated into 500-1600 μm and 1600-3500 μm size-classes, which was not done for plankton from the west coast. The $\delta^{13}C$ values of larger plankton become more positive than values for smaller plankton and there is less overlap between the data for the different size-classes of plankton than is the case for the west coast plankton, except for the 500-1600 μm and 1600-3500 μm size-fractions, which have similar means and standard deviations. The data for 200-500 μm plankton have significantly more positive $\delta^{13}C$ values than plankton in the 20-200 μm size-fraction. Furthermore, the $\delta^{13}C$ data for plankton in the 500-1600 μm size-fraction are significantly more positive than the data for 200-500 μm plankton. The 500-1600 μm and 1600-3500 μm size-classes are not significantly different (Table 5).

The $\delta^{15}N$ data obtained for the plankton caught from the west coast are shown in Fig. 17 (statistics in Table 6). As is the case for $\delta^{13}C$, the $\delta^{15}N$ values become more positive with increasing plankton size. The $\delta^{15}N$ values for 200-500 μm and >500 μm plankton are significantly larger than those for 20-200 μm plankton. The data for the 200-500 μm and >500 μm size classes are not however, significantly different. In contrast to the $\delta^{13}C$ results, the confidence intervals increase with increasing size-class. Thus it appears that scatter within the 20-200 μm plankton may not have a trophic basis as is suggested by the corresponding $\delta^{13}C$ results. Species composition may be important, since phytoplankton

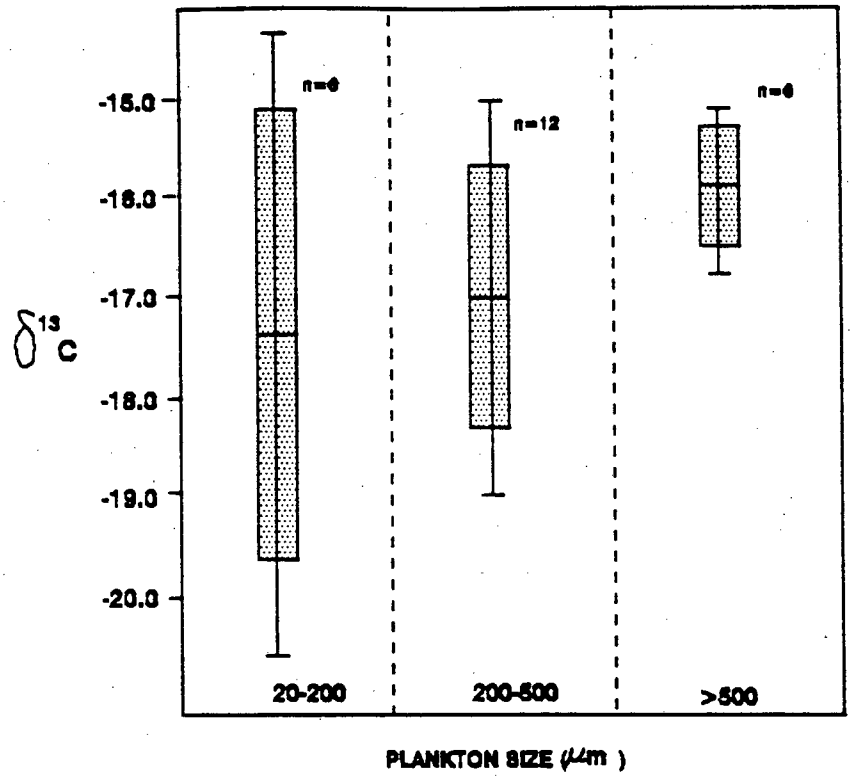


Fig. 15 $\delta^{13}C$ measurements for different size-classes of plankton caught from the west coast of South Africa. Vertical lines represent data ranges, rectangles represent standard deviations and the means are given by horizontal lines inside rectangles. Numbers of measurements (n) are also given.

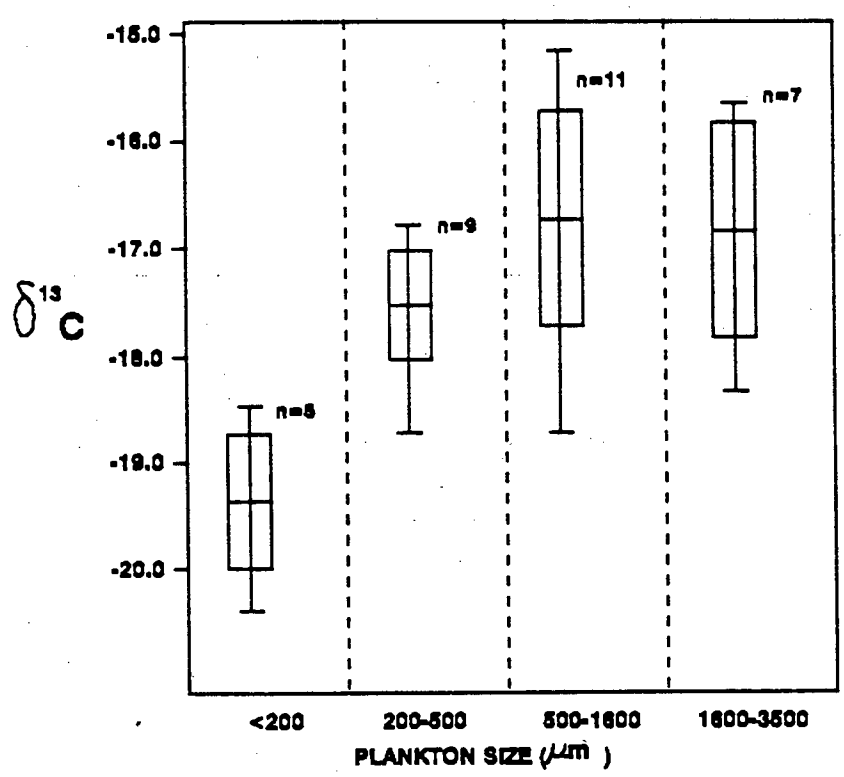


Fig. 16 $\delta^{13}C$ measurements for different size-classes of plankton caught from the Agulhas Bank area. Vertical lines represent data ranges, rectangles represent standard deviations and the means are given by horizontal lines inside rectangles. Numbers of measurements (n) are also given.

Table 5 Carbon isotope ratio statistics for plankton from the west coast of South Africa (southern Benguela ecosystem) and Agulhas Bank area, showing number of measurements, mean $\delta^{13}C$ values (n), their standard deviations (SD), the differences between successive categories (D), the standard error of the differences (SE) and the significant differences from Mann-Whitney U-tests (U = the test statistic for 1-tailed test, p = probability value for 1-tailed test) and Kruskal-Wallis one-way ANOVA (H = the test statistic, p = associated probability value), ns = not significant.

SOUTHERN BENGUELA

Category	n	Mean	SD	D (SE)	Significance
(20-200μm)	6	-17.3	(2.5)	-	
(200-500μm)	12	-17.0	(1.3)	0.3 (1.1)	ns
(>500μm)	6	-15.9	(0.6)	1.1 (0.5)	ns
Total increase <200μm to >500μm				1.4 (1.0)	ns

AGULHAS BANK

Category	n	Mean	SD	D (SE)	Significance	
(<200μm)	5	-19.4	(0.7)	-		
(200-500μm)	9	-17.6	(0.6)	1.8 (0.4)	U=44, p<0.001	H=17.1, p<0.001
(500-1600μm)	11	-16.7	(0.9)	0.9 (0.3)	U=83, p<0.05	
(1600-3500)	7	-16.8	(1.0)	0.1 (0.4)	ns	
Total increase <200μm to 1600-3500μm				2.6 (0.5)		

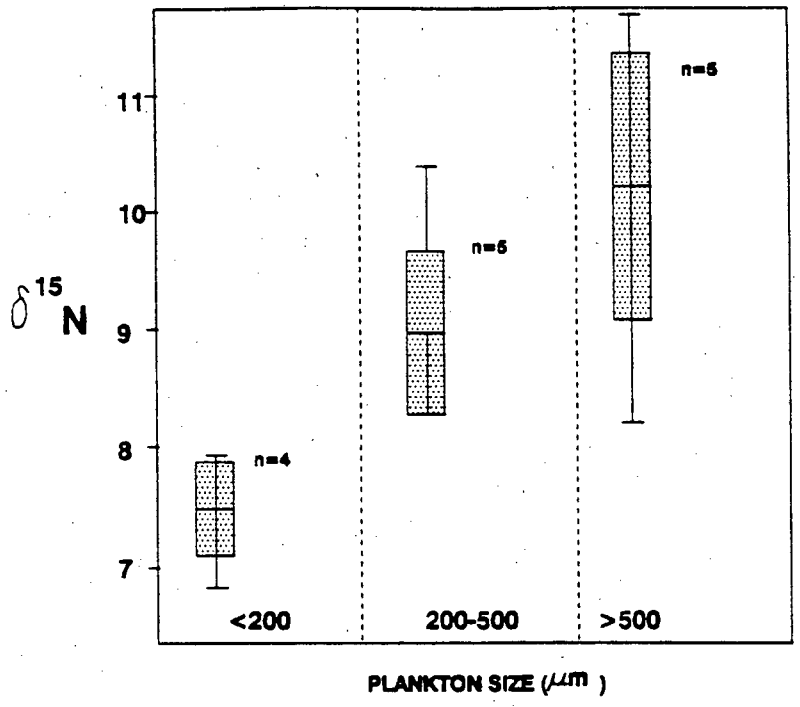


Fig. 17 $\delta^{15}N$ measurements for different size-classes of plankton caught from the west coast of South Africa. Vertical lines represent data ranges, rectangles represent standard deviations and the means are given by horizontal lines inside rectangles. Numbers of measurements (n) are also given.

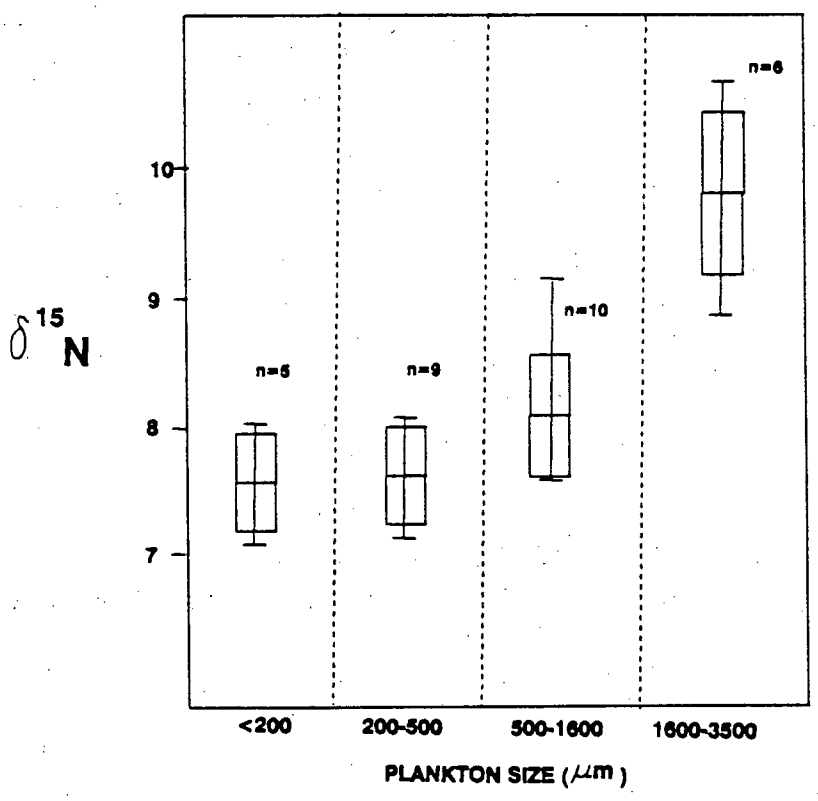


Fig. 18 $\delta^{15}N$ measurements for different size-classes of plankton caught from the Agulhas Bank area. Vertical lines represent data ranges, rectangles represent standard deviations and the means are given by horizontal lines inside rectangles. Numbers of measurements (n) are also given.

Table 6 Nitrogen isotope ratio statistics for plankton from the west coast of South Africa and Agulhas Bank area, showing number of measurements, mean $\delta^{15}N$ values, their standard deviations, differences between successive categories (D), the standard error of the differences (SE) and the significant differences from Mann-Whitney U-tests (U = the test statistic for 1-tailed test, p = probability value for 1-tailed test) and Kruskal-Wallis one-way ANOVA (H = the test statistic, p = the associated probability value), ns = not significant.

SOUTHERN BENGUELA

Category	n	Mean	SD	D (SE)	Significance	
(20-200μm)	4	7.5	(0.4)	-		
(200-500μm)	5	9.0	(0.8)	1.5 (0.4)	U=20, p<0.01	H=10.0, p<0.01
(>500μm)	6	10.2	(1.1)	1.2 (0.6)	ns	
Total increase <200μm to >500μm				2.7 (0.5)	U=24, p<0.005	

AGULHAS BANK

Category	n	Mean	SD	D (SE)	Significance	
(<200μm)	4	7.5	(0.3)	-		
(200-500μm)	9	7.7	(0.3)	0.2 (0.2)	ns	
(500-1600μm)	10	8.1	(0.5)	0.4 (0.2)	U=71, p<0.025	H=15.9, p<0.001
(1600-3500μm)	6	9.9	(0.6)	1.8 (0.3)	U=59, p<0.0005	
Total increase <200μm to 3500μm				2.4 (0.3)		

may fractionate $\delta^{13}C$ to different degrees according to their choice of photosynthetic pathway or a number factors related to their immediate environment (section 1.3.3).

Fig. 18 shows the $\delta^{15}N$ data for plankton from the Agulhas Bank region (statistics in Table 6). Once again the $\delta^{15}N$ values become more positive with increasing plankton size. The data for plankton in the <200 μm and 200-500 μm size-classes are not significantly different in $\delta^{15}N$. Data for plankton in the 500-1600 μm size-class are significantly more positive than those for 200-500 μm plankton and in contrast to the $\delta^{13}C$ data, the data for 1600-3500 μm plankton are significantly more positive than those for 500-1600 μm plankton. The standard deviations become larger with increasing plankton size-class.

For comparison Fig. 19 shows the $\delta^{13}C$ results obtained for plankton from both the west coast and Agulhas Bank areas on the same graph (statistics in Table 7). Plankton from the west coast include organisms with more positive $\delta^{13}C$ values in each size-class than from the Agulhas Bank. The mean $\delta^{13}C$ value for plankton in the <200 μm size-class from the west coast (-17.3 $^{o}/_{oo}$) is 2.1 $^{o}/_{oo}$ more positive than the mean for plankton from the Agulhas Bank (-19.4 $^{o}/_{oo}$). This is not a statistically significant difference. The mean $\delta^{13}C$ values for plankton in the 200-500 μm size-class from the west coast and Agulhas Bank areas (-17.0 $^{o}/_{oo}$ and -17.6 $^{o}/_{oo}$ respectively) are not significantly different. The $\delta^{13}C$ mean for >500 μm plankton from the west coast (-15.9 $^{o}/_{oo}$) is more positive, by ca. 0.8 $^{o}/_{oo}$, than the means for plankton from the Agulhas Bank in the 500-1600 μm (-16.7 $^{o}/_{oo}$) and 1600-3500 μm (-16.8 $^{o}/_{oo}$) size-classes. The >500 μm plankton from the west coast have significantly more positive $\delta^{13}C$ values than the data for 500-1600 μm plankton from the Agulhas Bank, but not significantly more positive than the 1600-3500 μm size-class. The >500 μm plankton from the west coast also have significantly more positive $\delta^{13}C$ values than the combined 500-3500 μm size-fraction of plankton from the Agulhas Bank (mean = -16.7 $^{o}/_{oo}$).

Fig. 20 shows the $\delta^{15}N$ results obtained for plankton from both the west coast and the Agulhas Bank areas (statistics in Table 7). As for $\delta^{13}C$, the results for plankton in each size-class from the west coast include values more positive $\delta^{15}N$ values than those for plankton from the Agulhas Bank, except for the <200 μm size class, where the data ranges and means for the west coast and Agulhas Bank plankton are similar.

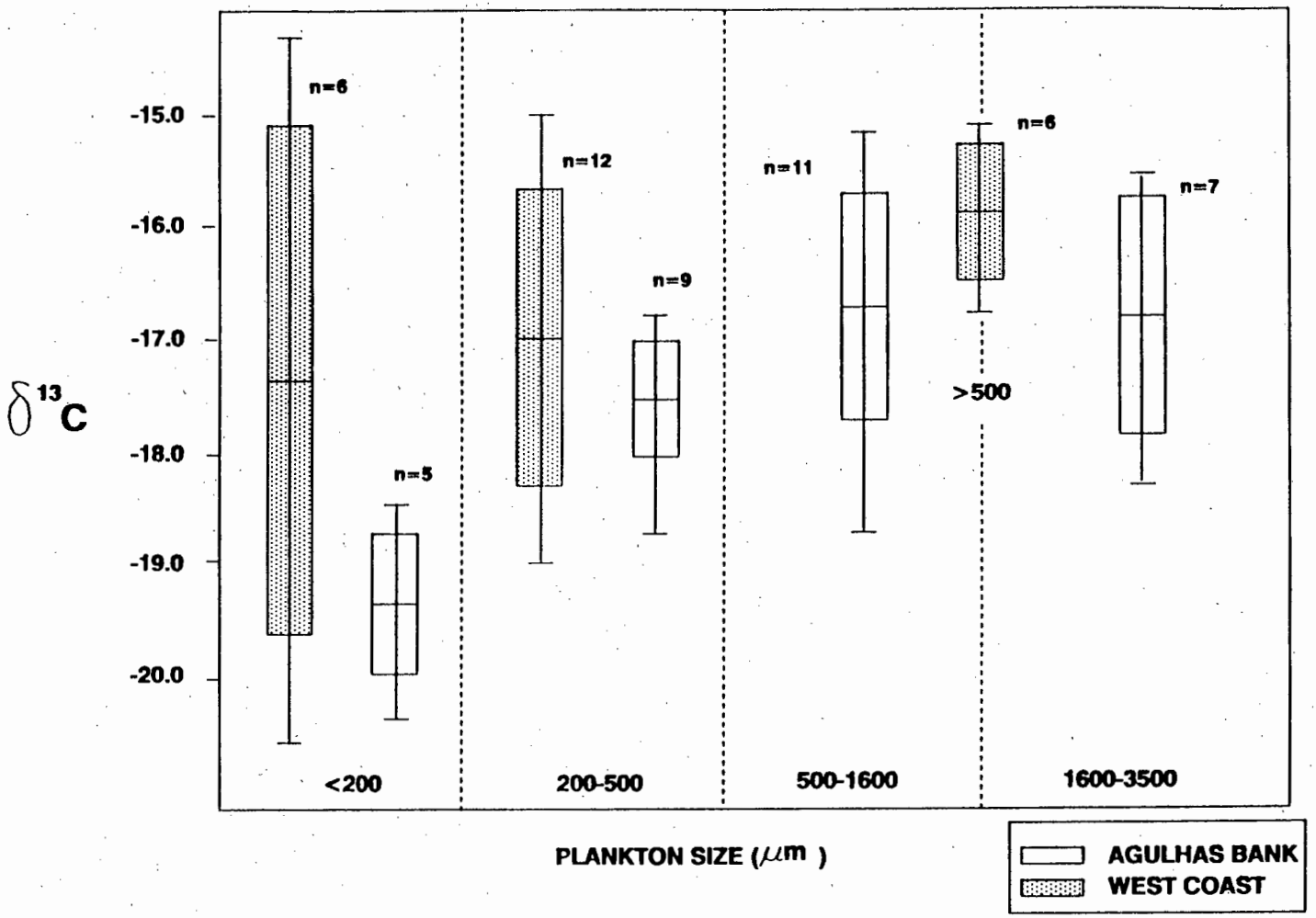


Fig. 19 $\delta^{13}C$ measurements for different size-classes of plankton. Those caught from the west coast and those caught from the Agulhas Bank area are represented on the same graph for comparison. Vertical lines represent data ranges, rectangles represent standard deviations and the means are given by horizontal lines inside rectangles. Numbers of measurements (n) are also given.

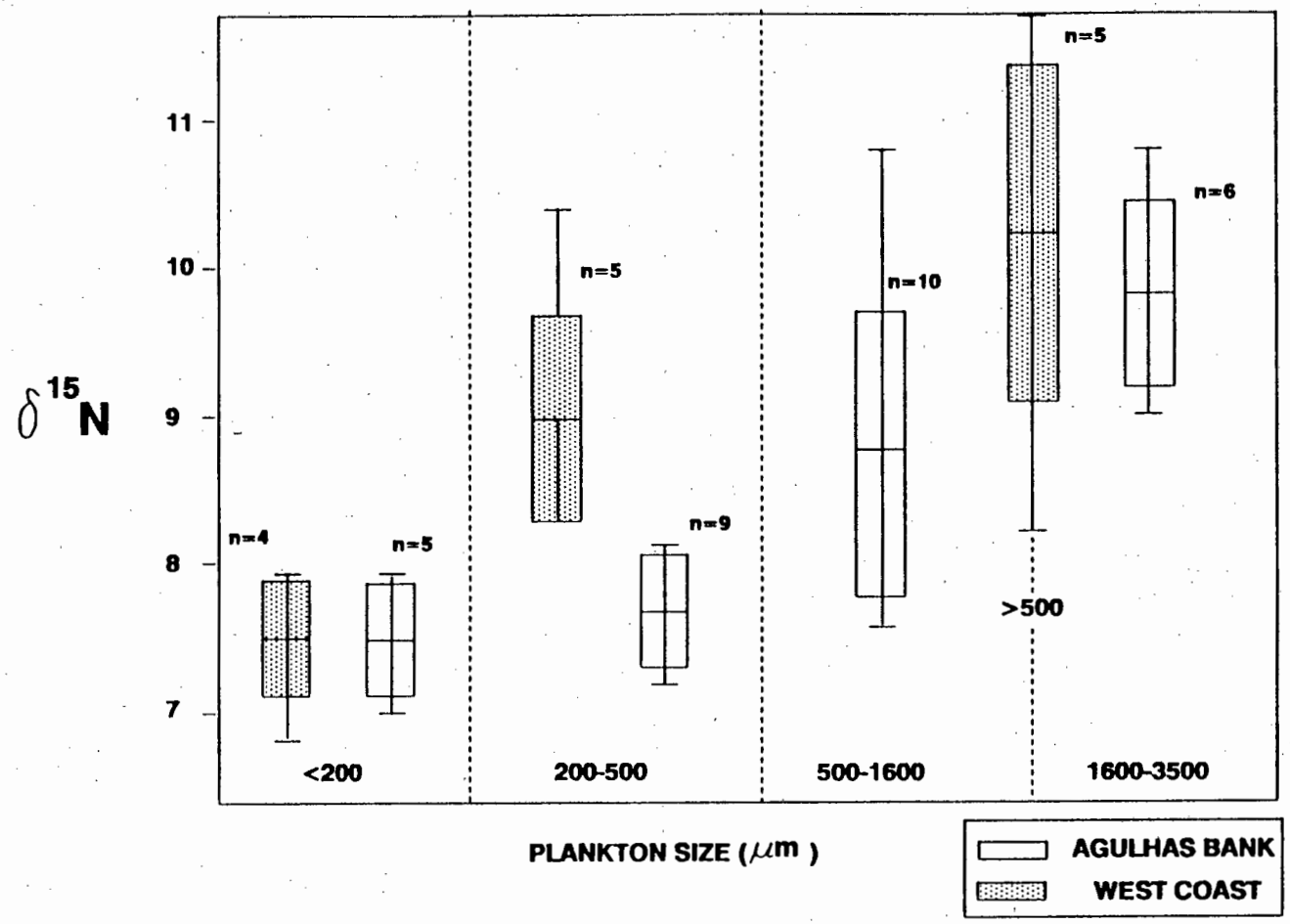


Fig. 20 $\delta^{15}N$ measurements for different size-classes of plankton. Those caught from the west coast and those caught from the Agulhas Bank are represented on the same graph for comparison. Vertical lines represent data ranges, rectangles represent standard deviations and the means are given by horizontal lines inside rectangles. Numbers of measurements (n) are also given.

Table 7: $\delta^{13}C$ and $\delta^{15}N$ statistics for plankton from the west coast of South Africa versus those from the Agulhas Bank, showing Mann-Whitney-U statistical tests (U = the test statistic for 2-tailed test, p = probability value for 2-tailed test) and Kruskal-Wallis one-way ANOVA (H = the test statistic, p = the associated probability value), ns = not significant

MAY 1988	NOVEMBER 1989	SIGNIFICANCE	
$\delta^{13}C$:			
<200 μm	vs <200 μm	ns	H=18.5, p<0.005
200-500 μm	vs 200-500 μm	ns	
>500 μm	vs 500-1600 μm	U=52, p<0.05	
	vs 1600-3500 μm	ns	
	vs 500-3500 μm	U=86, p<0.05	
$\delta^{15}N$:			
<200 μm	vs <200 μm	ns	H=31.1, p<0.00002
200-500 μm	vs 200-500 μm	U = 45, p<0.001	
>500 μm	vs 500-1600 μm	U = 56.5, p<0.005	
	vs 1600-3500 μm	ns	
	vs 500-3500 μm	U = 80, p<0.02	

The data for west coast and Agulhas Bank plankton in the 200-500 μm size-class do not overlap. The mean $\delta^{15}N$ value for 200-500 μm plankton from the west coast (9.0 $^{o}/_{oo}$) is 1.3 $^{o}/_{oo}$ more positive than the mean for plankton in this size-class from the Agulhas Bank (7.7 $^{o}/_{oo}$). The data for the plankton in this size-class from the two areas are significantly different.

The mean $\delta^{15}N$ value for plankton from the west coast in the >500 μm size-class (10.2 $^{o}/_{oo}$) is more positive than the mean for plankton from the Agulhas Bank in the 500-1600 μm size-class (8.1 $^{o}/_{oo}$) by 2.1 $^{o}/_{oo}$ and more positive than the mean for the 1600-3500 μm size-class (9.3 $^{o}/_{oo}$) by only 0.9 $^{o}/_{oo}$. The $\delta^{15}N$ values for west coast plankton in the >500 μm size class are significantly greater than the data for 500-1600 μm plankton from the Agulhas Bank (Table 7), but are not significantly different to the data for plankton in the 1600-3500 μm size-fraction. The $\delta^{15}N$ data for plankton in the >500 μm size-class from the west coast are also significantly larger than data for the combined 500-3500 μm size fraction of plankton from the Agulhas Bank.

The means for each size-class of plankton from the Agulhas Bank are close to those for the smaller size-class of plankton from the west coast (Figs. 19 and 20). Therefore it appears that plankton in each size-class from the Agulhas Bank, were consuming a food source of about one size class smaller than those from the west coast. Lack of an important trophic link amongst the plankton from the Agulhas Bank may, in part, be responsible for the less positive values of plankton in this region. It is possible that the season during which the plankton were caught is important (winter for the west coast and summer for the Agulhas Bank samples). Temperature is unlikely to be responsible for the more enriched values among the west coast plankton, since winter SST in the southern Benguela are likely to be lower than the average summer SST in the Agulhas Bank region (see section 1.4). Plankton from the Agulhas Bank were caught in surface waters, above the thermocline (see chapter 2). Therefore, we can expect the Agulhas Bank samples from this study to have been caught in warmer waters than the west-coast samples from the southern Benguela ecosystem, but they do not accordingly have more positive $\delta^{13}C$ values (as found by Fontugne & Duplessy 1981). Nutrient availability may be important, since the plankton from the west coast (southern Benguela ecosystem) were captured towards the end of the upwelling period (May 1988), a period of potentially high standing stock, and low nutrient conditions (Probyn 1985; Shannon & Field 1985). In terms of isotope

kinetics a decline in nutrient availability (the substrate) should result in decreased discrimination against the heavier isotopes and therefore, isotopic enrichment. Organisms higher up the foodweb would accordingly become more enriched. One must bear in mind, however, that the $\delta^{13}C$ and $\delta^{15}N$ means for plankton less than 200 μm in size (which contains phytoplankton) from the west coast are not significantly different to those for <200 μm plankton from the Agulhas Bank (Table 7). This may be explained by the fact that particulate excreta have been found to be more isotopically enriched than copepod bodies and ingested material (Checkley & Entzeroth 1985), while soluble metabolites are more isotopically depleted (CO_2 and NH_4^+). Hence as phytoplankton become increasingly dependent on recycled material, they may become depleted once more (due to uptake of ammonium, urea and recycled CO_2). Particulate consumers further up the foodweb would remain more isotopically enriched due to their increased dependance on recycled SPM (suspended particulate matter). Probyn (1985) has estimated that ca. 30 % of phytoplankton nitrogen uptake in Benguela surface waters over the continental shelf may be regenerated nitrogen, while in oceanic waters, regenerated nitrogen is the principle nitrogen source.

5.2 PLANKTON TO GUT CONTENTS

Fig. 21 shows the $\delta^{13}C$ results for the different size-classes of plankton and for the gut contents and tissues of the different fish species caught from the Benguela ecosystem off the west coast of South Africa. The $\delta^{13}C$ measurements for the gut contents of anchovy caught from the west coast are similar to those for the two smaller plankton size-classes caught from the same area, suggesting that most of the plankton consumed by these fish at the time of capture was <500 μm. The values for >500 μm plankton are significantly different from those for anchovy gut contents (Table 11). The $\delta^{13}C$ values for roundherring gut contents are significantly more negative than those for anchovy gut contents (Table 8). Roundherring gut contents have significantly more negative $\delta^{13}C$ values than both 200-500 μm and >500 μm plankton (Table 11). The few sardine gut contents measured have more negative $\delta^{13}C$ values than both anchovy (significant) and roundherring (not significant) (Table 8).

Results for the gut contents of 3 sardine from the Agulhas Bank region were obtained, but the stomachs of the other fish from this area were empty. The $\delta^{13}C$ values for the gut

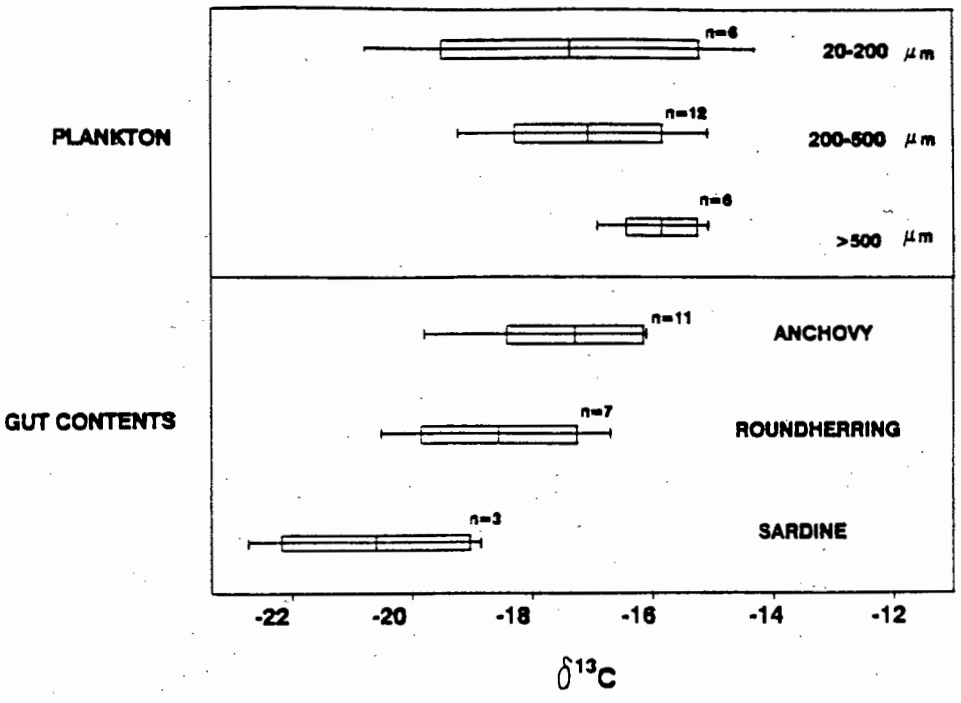


Fig. 21 $\delta^{13}C$ measurements for different size-classes of plankton and for the gut contents of Cape anchovy Engraulis capensis, roundherring Etrumeus whiteheadi and sardine Sardinops ocellatus, all from the west coast of South Africa. All size-classes of fish are represented in the gut content samples. Horizontal lines represent data ranges, rectangles represent standard deviations and the means are given by vertical lines inside rectangles. Numbers of measurements (n) are also given.

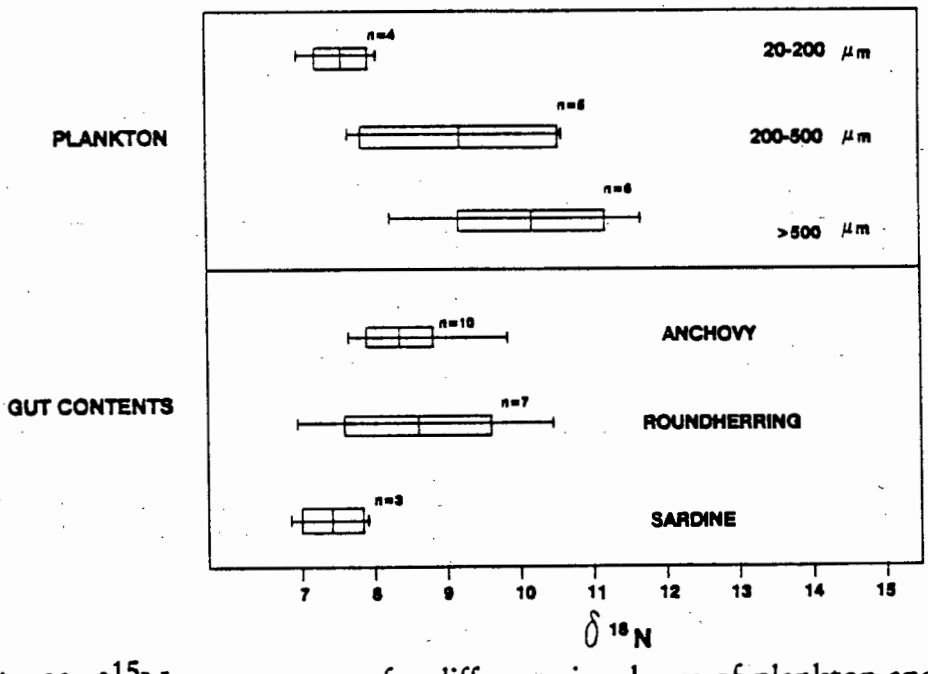


Fig. 22 $\delta^{15}N$ measurements for different size-classes of plankton and for the gut contents of Cape anchovy Engraulis capensis, roundherring Etrumeus whiteheadi and sardine Sardinops ocellatus, all from the west coast of South Africa. All size-classes of fish are represented in the gut content samples. Horizontal lines represent data ranges, rectangles represent standard deviations and the means are given by vertical lines inside rectangles. Numbers of measurements (n) are also given.

Table 8: $\delta^{13}C$ and $\delta^{15}N$ statistics for the gut contents of anchovy versus the gut contents of roundherring versus the gut contents of sardine, all from the west coast of South Africa, showing Mann-Whitney-U statistical tests (U = the test statistic for 2-tailed test, p = significant for 2-tailed test) and Kruskal-Wallis one-way ANOVA (H = the test statistic, p = the associated probability value), ns = not significant.

$\delta^{13}C$:

Anchovy gut	vs	Roundherring gut	ns	H=7.5, p<0.02
Anchovy gut	vs	Sardine gut	U=29, p<0.02	
Roundherring gut	vs	Sardine gut	ns	

$\delta^{15}N$:

Anchovy gut	vs	Roundherring gut	U=60, P<0.05	H=4.7, p<0.1
Anchovy gut	vs	Sardine gut	U=31, p<0.05	
Roundherring gut	vs	Sardine gut	ns	

contents of sardine from the Agulhas Bank (mean = -16.5 $^{o}/_{oo}$) are significantly more positive than the gut contents of sardine from the west coast (mean = -20.4 $^{o}/_{oo}$) ($U=9$, $p<0.05$) and significantly more positive than <200 μm plankton from the same area (Table 11).

Fig. 22 shows the $\delta^{15}N$ results for the different size-classes of plankton and for the gut contents and tissues from the different fish species caught from the west coast (southern Benguela ecosystem). The $\delta^{15}N$ data for anchovy gut contents are significantly more negative than >500 μm plankton as is the case for the $\delta^{13}C$ data (Table 13). Furthermore, the $\delta^{15}N$ values for anchovy gut contents are significantly more positive than those for 20-200 μm plankton (Table 13), so it is possible that small (20-200 μm) and large (>500 μm) plankton formed very little of the diet of west coast anchovy when caught. Roundherring and anchovy gut contents are not significantly different in $\delta^{15}N$, as was the case for $\delta^{13}C$ (Table 8). Sardine gut contents tend to have more negative $\delta^{15}N$ values than anchovy gut contents (significant) and roundherring gut contents (not significant) (Table 8). Sardine were probably consuming greater proportions of phytoplankton than the other two species of fish.

In contrast to the results for $\delta^{13}C$, the mean $\delta^{15}N$ values of the gut contents of sardine from the west coast and Agulhas Bank areas are similar (7.1 and 7.4 $^{o}/_{oo}$ respectively). The gut contents of sardine from the Agulhas Bank are similar in $\delta^{15}N$ to <200 μm and 200-500 μm plankton and have significantly more negative $\delta^{15}N$ values than 500-1600 μm and 1600-3500 μm plankton (Table 8).

5.3 FRACTIONATION IN FISH TISSUES

Anchovy caught from the Agulhas Bank area were between 110mm and 130mm in size (caudal length), which represent anchovy just over a year old (Waldron et al. 1989). The sardine caught from the same area were between 120mm and 210mm caudal length (between 8 months and 2 years), most of which were 170mm and therefore less than 1.5 years old (Waldron pers. comm.). The size, position and month (November) both species of fish were caught from the Agulhas Bank area suggest that they represent fish that have recently moved from the west coast to the south and east to spawn. Hence it is unlikely that their muscle tissue would isotopically reflect plankton from the eastern Agulhas

Bank. Nevertheless the relationships between plankton and fish are explored separately for each area to eliminate possible variations caused by differences in the two environments.

- Gut contents versus fish tissues:

The tissues of each species of fish from the west coast (southern Benguela) have more positive $\delta^{13}C$ and $\delta^{15}N$ values than their corresponding gut content values (Figs 23 and 24). The $\delta^{13}C$ difference between gut contents and tissues is similar for anchovy and roundherring. Thus roundherring tissues tend to have slightly more negative $\delta^{13}C$ values than anchovy tissues, as is the case for their gut contents. The $\delta^{13}C$ difference from gut contents to tissues for anchovy and roundherring (estimated from mean values) is of the order of 2.1-2.3 $^{o}/_{oo}$ respectively, for muscle, and 3.2-3.9 $^{o}/_{oo}$ for bone collagen (Table 9). As is pointed out in chapters 3 and 4, bone collagen tends to have more positive $\delta^{13}C$ values than muscle tissue. The difference between the mean values for anchovy muscle and bone collagen is 1.1 $^{o}/_{oo}$ (significantly different) and 1.6 $^{o}/_{oo}$ for round-herring tissues (not statistically significant) (Table 9). The degree of ^{13}C enrichment from diet (as gut contents at capture) to anchovy or roundherring muscle tissue is comparable to the data from Lee-Thorp et al. (1989), but the bone collagen tissues of anchovy and roundherring show slightly less enrichment than muscle tissue. The isotopic make-up of some of their juvenile diet may still be reflected in the bone collagen tissue (smaller fish probably consume smaller, less isotopically positive, plankton), which reflects a longer dietary history than muscle tissue. Sardine show a greater $\delta^{13}C$ difference between gut contents and muscle or bone collagen tissues (5.5 $^{o}/_{oo}$ or 6.6 $^{o}/_{oo}$ respectively) than diet-consumer relationships reported in the literature. Their gut contents are probably not a good reflection of their true diet since these gut-tissue differences exceed the diet-consumer differences reported in the literature. The difference between their muscle and bone collagen tissues is, however, similar to the other two fish species (1.1 $^{o}/_{oo}$). The average $\delta^{13}C$ content of whole animals is likely to be weighted towards the isotopic make-up of muscle tissue, since muscle tissue makes up a larger part of fish mass. Furthermore the inclusion of body organs and lipids would yield less positive values for consumers (De Niro & Epstein 1978). Thus a diet-consumer difference of <2 $^{o}/_{oo}$, as is reported in the literature for whole bodies of animals, seems likely (De Niro & Epstein 1978; Rau et al. 1983).

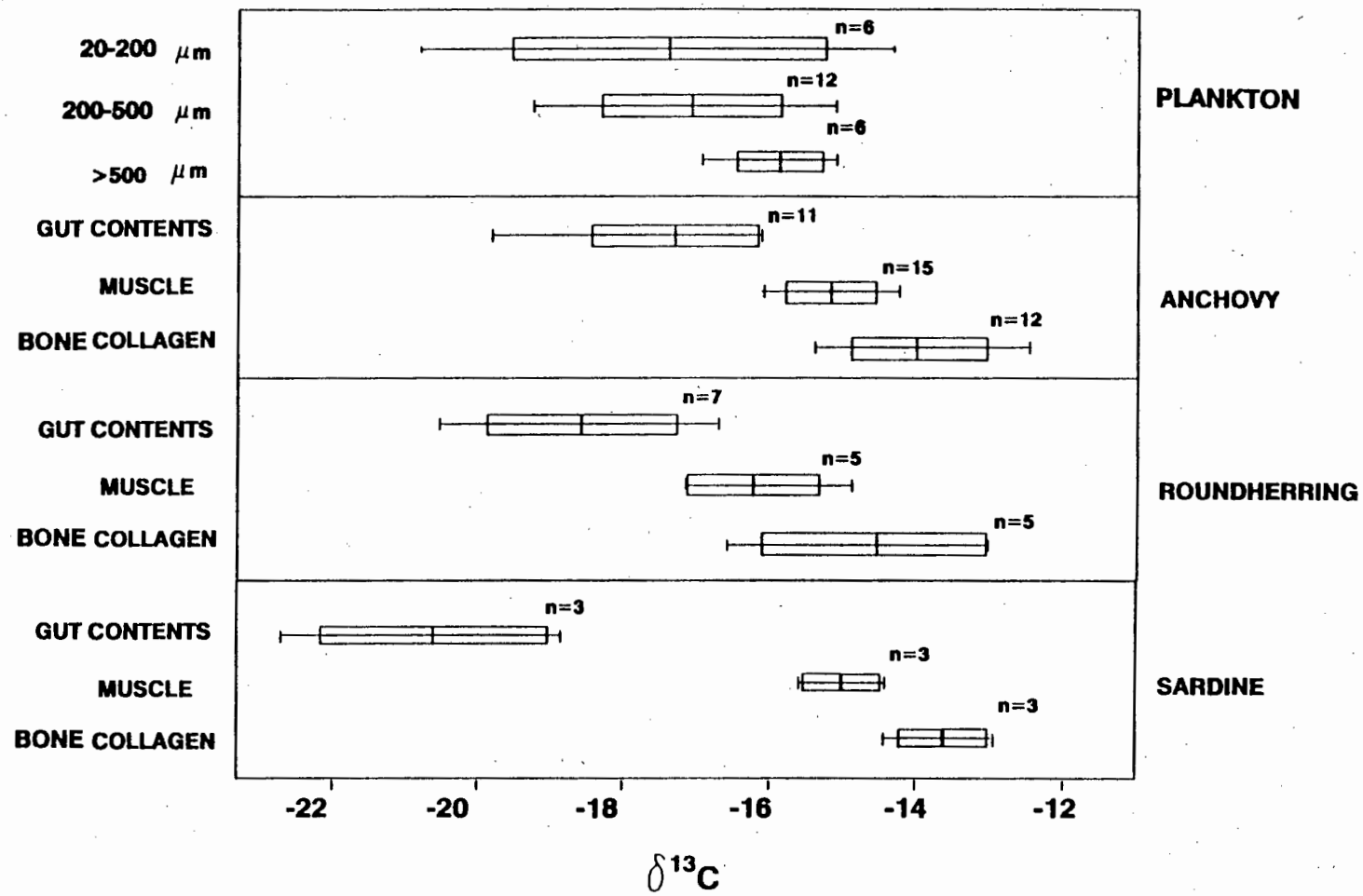


Fig. 23 $\delta^{13}C$ measurements for different size-classes of plankton and for the gut contents, muscle and bone collagen tissues of Cape anchovy Engraulis capensis, roundherring Etrumeus whiteheadi and sardine Sardinops ocellatus, all from the west coast of South Africa. All size-classes of fish are represented in the gut content and tissue samples. Horizontal lines represent data ranges, rectangles represent standard deviations and the means are given by vertical lines inside rectangles. Numbers of measurements (n) are also given.

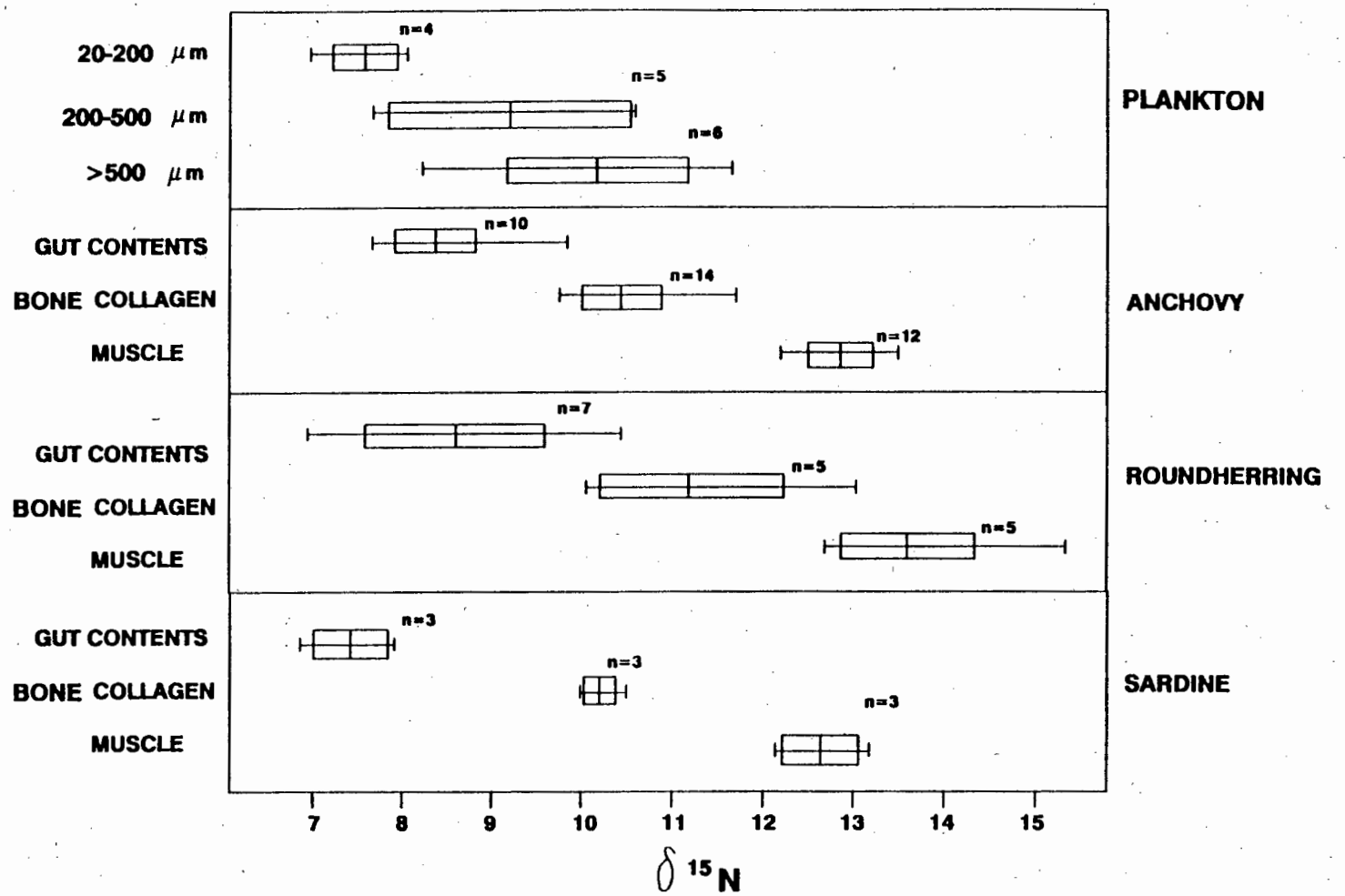


Fig. 24 $\delta^{15}N$ measurements for different size-classes of plankton and for the gut contents, muscle and bone collagen tissues of Cape anchovy Engraulis capensis, roundherring Etrumeus whiteheadi and sardine Sardinops ocellatus, all from the west coast of South Africa. All size-classes of fish are represented in the gut content and tissue samples. Horizontal lines represent data ranges, rectangles represent standard deviations and the means are given by vertical lines inside rectangles. Numbers of measurements (n) are also given.

Table 9 Carbon isotope ratio statistics for anchovy Engraulis capensis, roundherring, Etrumeus whiteheadi and sardine, Sardinops ocellatus from the west coast of South Africa, showing number of measurements, mean $\delta^{13}C$ values, their standard deviations, differences between successive categories (D), the standard error of the differences (SE) and the significant differences from Mann-Whitney U-tests (U = the test statistic for 1-tailed test, p = probability value for 1-tailed test) and Kruskal-Wallis one-way ANOVA (H = the test statistic, p = the associated probability value), ns = not significant.

ANCHOVY	n	mean	SD	D (SE)	Significance	
Gut contents	11	-17.2	(1.1)	-		
Muscle	14	-15.1	(0.6)	2.1 (0.4)	U=153, p<0.0005	H=26.5, p<0.000002
Bone	12	-14.0	(0.9)	1.1 (0.3)	U=139, p<0.0025	
Total enrichment (gut to bone)				3.2 (0.4)		
ROUNDHERRING						
Gut contents	7	-18.5	(1.4)	-		
Muscle	5	-16.2	(0.9)	2.3 (0.6)	U=32, p<0.01	H=11.3, p<0.005
Bone	5	-14.6	(1.6)	1.6 (0.8)	U=21, p<0.05	
Total enrichment (gut to bone)				3.9 (0.9)		
SARDINE						
Gut contents	3	-20.4	(1.7)			
Muscle	3	-14.9	(0.4)	5.5 (1.0)	U=9, p<0.05	H=7.2, p<0.05
Bone	3	-13.8	(0.5)	1.1 (0.4)	U=9, p<0.05	
Total enrichment (gut to bone)				6.6 (1.0)		

Table 10 Nitrogen isotope ratio statistics for anchovy Engraulis capensis, roundherring, Etrumeus whiteheadi and sardine, Sardinops ocellatus, all from the west coast of South Africa showing number of measurements (n), mean $\delta^{15}N$ values, their standard deviations (SD), differences between successive categories listed (D), the standard error of the differences (SE) and the significant differences from Mann-Whitney U-tests (U = the test statistic for 1-tailed test, p = probability value for 1-tailed test) and Kruskal-Wallis one-way ANOVA (H = the test statistic, p = the associated probability value), ns = not significant.

Category	n	Mean	SD	D (SE)	Significance	
ANCHOVY						
Gut contents	10	8.6	(0.7)	-		
Bone	12	10.6	(0.5)	2.0 (0.3)	U=119, p<0.0005	H=30.7, p<0.0000002
Muscle	14	12.9	(0.4)	2.3 (0.2)	U=168, p<0.0005	
Total increase (gut to muscle)				4.3 (0.2)		
ROUNDHERRING						
Gut contents	7	8.8	(1.2)	-		
Bone	5	11.3	(1.1)	2.5 (0.7)	U=32, p<0.01	H=12.0, p<0.002
Muscle	5	13.7	(0.8)	2.4 (0.6)	U=23, p<0.025	
Total increase (gut to muscle)				4.9 (0.6)		
SARDINE						
Gut contents	3	7.4	(0.3)			
Bone	3	10.2	(0.1)	2.8 (0.2)	U=9, p<0.05	H=7.2, p<0.05
Muscle	3	12.7	(0.4)	2.5 (0.3)		
Total increase (gut to muscle)				5.3 (0.2)		

The $\delta^{15}N$ difference between gut contents and bone collagen is of the order of 2.0 $^{o}/_{oo}$ to 2.5 $^{o}/_{oo}$ for anchovy and roundherring, respectively and 4.3 $^{o}/_{oo}$ to 4.9 $^{o}/_{oo}$ between gut contents and muscle tissue (Table 10). In the case of $\delta^{15}N$, muscle has more positive values than bone collagen tissue (see chapter 4). The differences between the means for muscle and bone collagen tissues are 2.3 $^{o}/_{oo}$ and 2.4 $^{o}/_{oo}$ for anchovy and roundherring respectively (statistically significant, Table 10). Although the gut contents/tissue disparity is slightly greater for roundherring than anchovy, the bone collagen/muscle disparity for both species is the same (their tissues are similar in $\delta^{15}N$). In contrast to the $\delta^{13}C$ results, sardine shows a similar $\delta^{15}N$ difference between their gut contents and tissues (2.8 $^{o}/_{oo}$ for bone collagen and 5.3 for muscle tissue) and between their bone collagen and muscle tissues (2.5 $^{o}/_{oo}$) as do the other fish species.

The average $\delta^{15}N$ content of whole fish, weighted towards the abundant muscle tissue, would result in a $\delta^{15}N$ difference in the region of 4 to 5 $^{o}/_{oo}$. Again this is consistent with values reported in the literature (De Niro & Epstein 1981; Minagawa & Wada 1984).

- Plankton versus fish tissues :

The tissues of all species of fish have more positive $\delta^{13}C$ and $\delta^{15}N$ values than all size classes of plankton to (Figs. 23, 24). Thus the fish may have previously consumed plankton of any size despite what was present in their gut contents at the time of capture. It is not possible to estimate the proportions different plankton size classes contribute to their diet with these data alone. If one is to substitute isotope analyses for more conventional gut content analyses, it is important to determine how the tissues themselves compare isotopically, with the different size-classes of plankton.

The muscle tissue of anchovy from the west coast has significantly more positive $\delta^{13}C$ values than 200-500 μm and >500 μm plankton from the same area (Table 11). One may therefore assume that either or both 200-500 μm and >500 μm plankton are consumed by anchovy. However, the $\delta^{13}C$ diet-consumer disparity (taken from mean values) between 200-500 μm plankton and anchovy muscle is ca. 2 $^{o}/_{oo}$ (Table 9). A disparity larger than this is unlikely for consumer muscle tissue. Hence the conclusion is similar to that evidenced by their gut contents i.e. that anchovy consume mostly 200-500 μm plankton.

Table 11: $\delta^{13}C$ statistics for the gut contents and tissues of anchovy, roundherring and sardine from the west coast of South Africa versus all size-classes of plankton from the same area, showing significant differences from Mann-Whitney-U tests (U = the test statistic for 1-tailed test, p = probability value for 1-tailed test) and Kruskal-Wallis one-way ANOVA (H = the test statistic, p = the associated probability value), ns = not significant.

ANCHOVY	VS PLANKTON	SIGNIFICANCE	
Gut contents	vs 20-200 μm	ns	
	vs 200-500 μm	ns	
	vs >500 μm	U=56, p<0.05	
Muscle	vs 20-200 μm	ns	
	vs 200-500 μm	U=152, p<0.0005	H=36.8, p<0.000001
	vs >500 μm	U=65, p<0.025	
Bone collagen	vs 20-200 μm	U=65, p<0.0025	
	vs 200-500 μm	U=140, p<0.0005	
	vs >500 μm	U=70, p<0.0005	

ROUND	VS PLANKTON	SIGNIFICANCE	
Gut contents	vs 20-200 μm	ns	
	vs 200-500 μm	U=66, p<0.05	
	vs >500 μm	U=41, p<0.005	H=13.5, p<0.02
Muscle	vs 20-200 μm	ns	
	vs 200-500 μm	ns	
	vs >500 μm	ns	
Bone collagen	vs 20-200 μm	ns	
	vs 200-500 μm	U=48.5, p<0.05	
	vs >500 μm	ns	

SARDINE	VS PLANKTON	SIGNIFICANCE	
Gut contents	vs 20-200 μm	ns	
	vs 200-500 μm	U=34, p<0.02	
	vs >500 μm	U=18, p<0.05	
Muscle	vs 20-200 μm	ns	
	vs 200-500 μm	U=34, p<0.01	
	vs >500 μm	U=16.5 p<0.05	H=16.5, p<0.005
Bone collagen	vs 20-200 μm	U=17, p<0.025	
	vs 200-500 μm	U=36, p<0.0025	
	vs >500 μm	U=18, p<0.025	

Table 12 $\delta^{13}C$ statistics for the tissues of anchovy versus the tissues of roundherring versus the tissues of sardine caught from the west coast of South Africa, showing significant differences from Mann-Whitney-U tests (U = the test statistic for 2-tailed test, p = probability value for 2-tailed test) and Kruskal-Wallis one-way ANOVA (H = the test statistic, p = the associated probability value), ns = not significant.

MUSCLE				
Anchovy	vs	Roundherring	U = 53.5, $p<0.02$	H=5.4, $p<0.1$
Anchovy	vs	Sardine	ns	
Roundherring	vs	Sardine	ns	
BONE COLLAGEN				
Anchovy	vs	Roundherring	ns	
Anchovy	vs	Sardine	ns	
Roundherring	vs	Sardine	ns	

It is important to be aware that the isotopic fractionation associated with fish length discussed in chapter 4, may tend to increase the scatter in the pooled tissue data for different sized fish, thus hampering statistical tests for tissue-diet differences. Anchovy bone collagen has significantly more positive $\delta^{13}C$ values than all size classes of plankton (Table 11). The difference between this tissue and the two smaller plankton size-classes are, however, within the 3-5 $^{o}/_{oo}$ disparity reported in the literature, supporting the suggestion that the amount of >500 μm plankton consumed by anchovy is negligible (unless in combination with smaller plankton).

The $\delta^{13}C$ diet-consumer disparity between 200-500 μm plankton and roundherring muscle is only ca. 0.8 $^{o}/_{oo}$, 1.2 $^{o}/_{oo}$ less than for anchovy muscle (Table 9). Thus these fish may, on average, consume more <200 μm plankton than anchovy. There is a significant difference between the $\delta^{13}C$ values for anchovy and roundherring muscle tissues but this is not the case for their bone collagen tissues (Table 11). Roundherring tissues do not have significantly more positive $\delta^{13}C$ values than plankton of any size class, except for bone collagen which is significantly more enriched than 200-500 μm plankton (Table 11).

The muscle and bone collagen tissues of sardine are not significantly different in $\delta^{13}C$ to the corresponding tissues of the other fish species (Table 12). Hence one cannot assume that they consume more <200 μm plankton on average as is evidenced by their gut content data. The tissues of all three fish species are statistically very similar in $\delta^{13}C$.

Anchovy bone collagen has significantly more positive $\delta^{15}N$ values 20-200 μm and 200-500 μm plankton but not >500 μm plankton (Table 13). One may therefore exclude >500 μm plankton from the diet (as was deduced from their gut contents values). Anchovy muscle tissue however, has significantly more positive $\delta^{15}N$ values than all size classes of plankton (Table 13).

Roundherring bone collagen tissue does not have significantly more positive $\delta^{15}N$ values than >500 μm plankton, as was the case for anchovy, but has significantly more positive $\delta^{15}N$ values than the two smaller plankton size-classes (Table 13). Hence they do not appear to consume plankton >500 μm in size. Roundherring muscle tissue is significantly more positive $\delta^{15}N$ values than all size-classes of plankton (Table 13).

Table 13: $\delta^{15}N$ statistics for the gut contents and tissues of anchovy (Engraulis capensis), roundherring (Etrumeus whiteheadi) and sardine (Sardinops ocellatus) from the west coast of South Africa versus all size-classes of plankton from the same area, showing Mann-Whitney-U tests (U = the test statistic for 2-tailed test, p = probability value for 2-tailed test, *p = 1-tailed probability value given if 2-tailed is not significant) and Kruskal-Wallis one-way ANOVA (H = the test statistic, p = the associated probability value), ns = not significant.

ANCHOVY	VS PLANKTON	SIGNIFICANCE	
Gut contents	vs <200 μm	U = 37.5, p<0.02,	
	vs 200-500 μm	ns	
	vs >500 μm	U = 52.5, p<0.02,	
Muscle	vs <200 μm	U=56, p<0.0005	H=43,0 p<0.00000005
	vs 200-500 μm	U=70, p<0.0005	
	vs >500 μm	U=78, p<0.0005	
Bone collagen	vs <200 μm	U=48, p<0.001	
	vs 200-500 μm	U=53.5, p<0.01	
	vs >500	ns	

ROUND	VS PLANKTON	SIGNIFICANCE	
Gut contents	vs <200 μm	U = 24.5, *p<0.05	
	vs 200-500 μm	ns	
	vs >500 μm	ns	
Muscle	vs <200 μm	U=20, p<0.01	H=23.4 p<0.0005
	vs 200-500 μm	U=25, p<0.005	
	vs >500 μm	U=30, p<0.0025	
Bone collagen	vs <200 μm	U=20, p<0.01	
	vs 200-500 μm	U=23, p<0.025	
	vs >500 μm	ns	

SARDINE	VS PLANKTON	SIGNIFICANCE	
Gut contents	vs <200 μm	ns	
	vs 200-500 μm	U = 15, p<0.05	
	vs >500 μm	U = 18, p<0.05	
Muscle	vs <200 μm	U=12, p<0.05	H=19.1 p<0.002
	vs 200-500 μm	U=15, p<0.025	
	vs >500 μm	U=15, p<0.025	
Bone collagen	vs <200 μm	U=12, p<0.05	
	vs 200-500 μm	ns	
	vs >500 μm	ns	

Table 14 $\delta^{15}N$ statistics for the tissues of anchovy versus the tissues of roundherring versus the tissues of sardine caught from the west coast of South Africa, showing significant differences from Mann-Whitney-U tests (U = the test statistic for 2-tailed test, p = probability value for 2-tailed test) and Kruskal-Wallis one-way ANOVA (H = the test statistic, p = the associated probability value), ns = not significant.

MUSCLE

Anchovy	vs	Roundherring	ns
Anchovy	vs	Sardine	ns
Roundherring	vs	Sardine	ns

BONE COLLAGEN

Anchovy	vs	Roundherring	ns
Anchovy	vs	Sardine	ns
Roundherring	vs	Sardine	ns

There are no significant differences in $\delta^{15}N$ between the corresponding tissues for all the fish species (Table 14). Thus, as for $\delta^{13}C$, the $\delta^{15}N$ data for sardine tissues show no suggestion that their average diet is isotopically more negative than that of the other two fish species.

Fig. 25 draws together the paired data for those fish from the west coast for which both $\delta^{13}C$ and $\delta^{15}N$ was measured for the same samples. A clear pattern emerges which overrides any species specific differences. The pooled values for gut contents, fish muscle and bone collagen tissues of all three species of fish, are distinct from one another (statistically significant, $H_c = 48.32$, $p < 0.001$ for $\delta^{13}C$; $H_c = 42.64$, $p < 0.001$ for $\delta^{15}N$). Following Kruskal-Wallis one-way ANOVA, nonparametric Tukey-type multiple comparisons were carried out to determine between which groups significant differences occur. There are significant differences in both $\delta^{13}C$ and $\delta^{15}N$ ratios between gut contents and bone collagen, bone collagen and muscle tissue and between gut contents and muscle tissue (Tables 15 and 16). This reveals a clear pattern of trophic enrichment in the heavier isotopes from the gut contents to the tissues of all three fish species.

Fig. 26 shows the $\delta^{13}C$ data for plankton and fish muscle from the Agulhas Bank area. The results for west coast anchovy and sardine muscle are represented on the same graph for comparison. Fig. 27 shows the corresponding $\delta^{15}N$ data. As for the plankton results, the mean $\delta^{13}C$ and $\delta^{15}N$ values for the muscle tissue of anchovy from the Agulhas Bank (-16.1 $^{o}/_{oo}$ and 12.5 $^{o}/_{oo}$) are more negative than those for anchovy from the west coast (-15.1 $^{o}/_{oo}$ and 12.9 $^{o}/_{oo}$), but there is much overlap between the data for anchovy muscle from the two areas. In this case the lack of relatively more positive values for anchovy from the Agulhas Bank is due to the fact that they were all larger than 110 mm caudal length (see chapter 4). The mean $\delta^{13}C$ and $\delta^{15}N$ values for the muscle of sardine from the Agulhas Bank (-15.9 $^{o}/_{oo}$ and 11.2 $^{o}/_{oo}$) are also more negative than those for sardine from the west coast (-14.9 $^{o}/_{oo}$ and 12.7 $^{o}/_{oo}$). There is a greater difference than that shown by anchovy and there is no overlap between the $\delta^{15}N$ data for the muscle tissue of sardine from the two areas (Fig. 27). The data are too few to assume that this is a size-related effect. The $\delta^{13}C$ results for the muscle tissue of Agulhas Bank sardine and anchovy are similar, as was the case for these species of fish from the west coast. However Fig. 27 shows that the muscle tissue of sardine from the Agulhas Bank has more negative

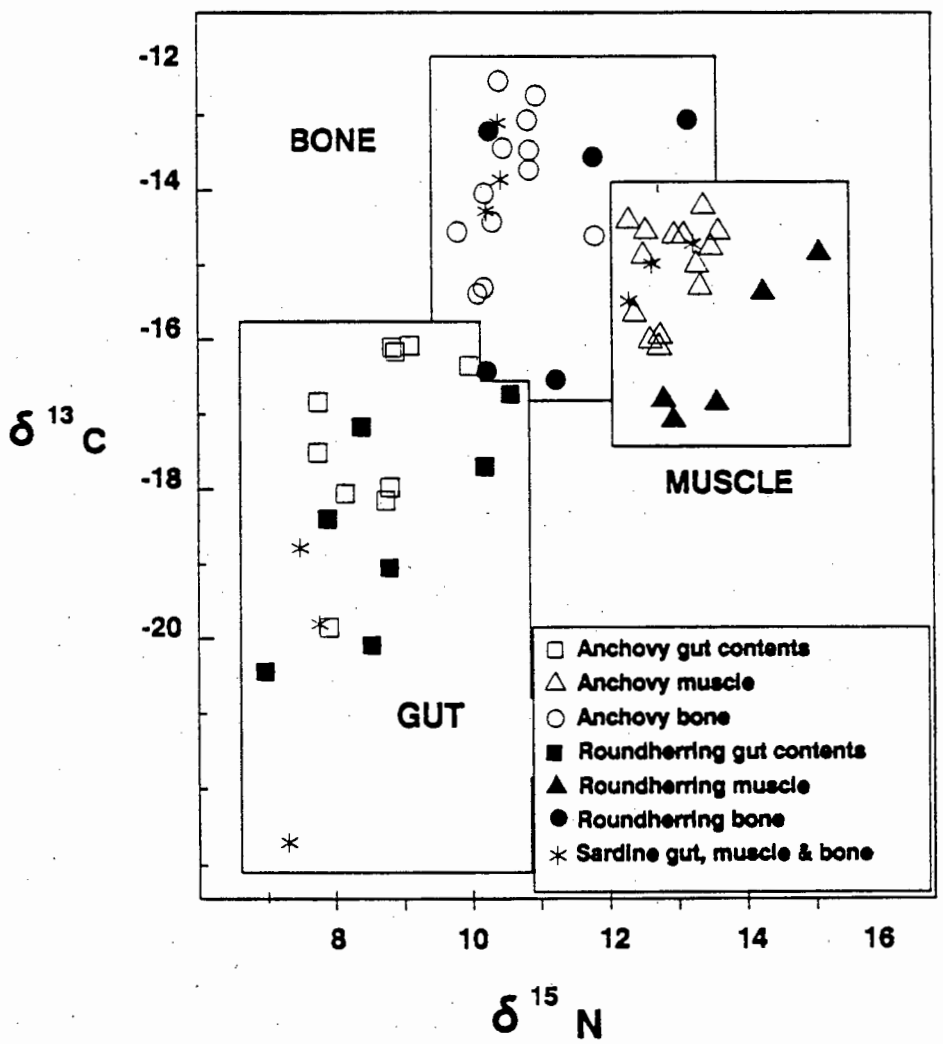


Fig. 25 $\delta^{13}C$ versus $\delta^{15}N$: Pooled values for the gut contents, muscle and bone collagen tissues of anchovy, roundherring and sardine (Engraulis capensis, Etrumeus whiteheadi and Sardinops ocellatus) from the west coast of South Africa.

TABLE 15

Results from a non-parametric multiple comparison test (Tukey type) to detect the differences between the pooled $\delta^{13}C$ results for anchovy and roundherring gut contents (G), muscle tissue (M) and bone collagen tissue (B). DIFF. = the difference between the mean ranks (rank sum/sample size), S.E. = the standard error (corrected for tied ranks), Q = the test statistic, $p < 0.05$.

COMPARISON	DIFF.	S.E.	Q	$Q_{0.05, 3}$	CONCLUSION
G vs. M	19.2	4.3	4.4	2.394	Reject H_0
G vs. B	30.6	4.5	6.9	2.394	Reject H_0
M vs. B	11.4	4.4	2.6	2.394	Reject H_0

TABLE 16

Results from a non-parametric multiple comparison test (Tukey type) to detect the differences between the pooled $\delta^{15}N$ results for anchovy and roundherring gut contents (G), muscle tissue (M) and bone collagen tissue (B). DIFF. = the difference between the mean ranks (rank sum/sample size), S.E. = the standard error (corrected for tied ranks), Q = the test statistic.

COMPARISON	DIFF.	S.E.	Q	$Q_{0.05, 3}$	CONCLUSION
G vs M.	33.6	5.2	6.5	2.394	Reject H_0
G vs. B	16.0	5.3	3.02	2.394	Reject H_0
B vs. M	17.6	5.2	3.4	2.394	Reject H_0

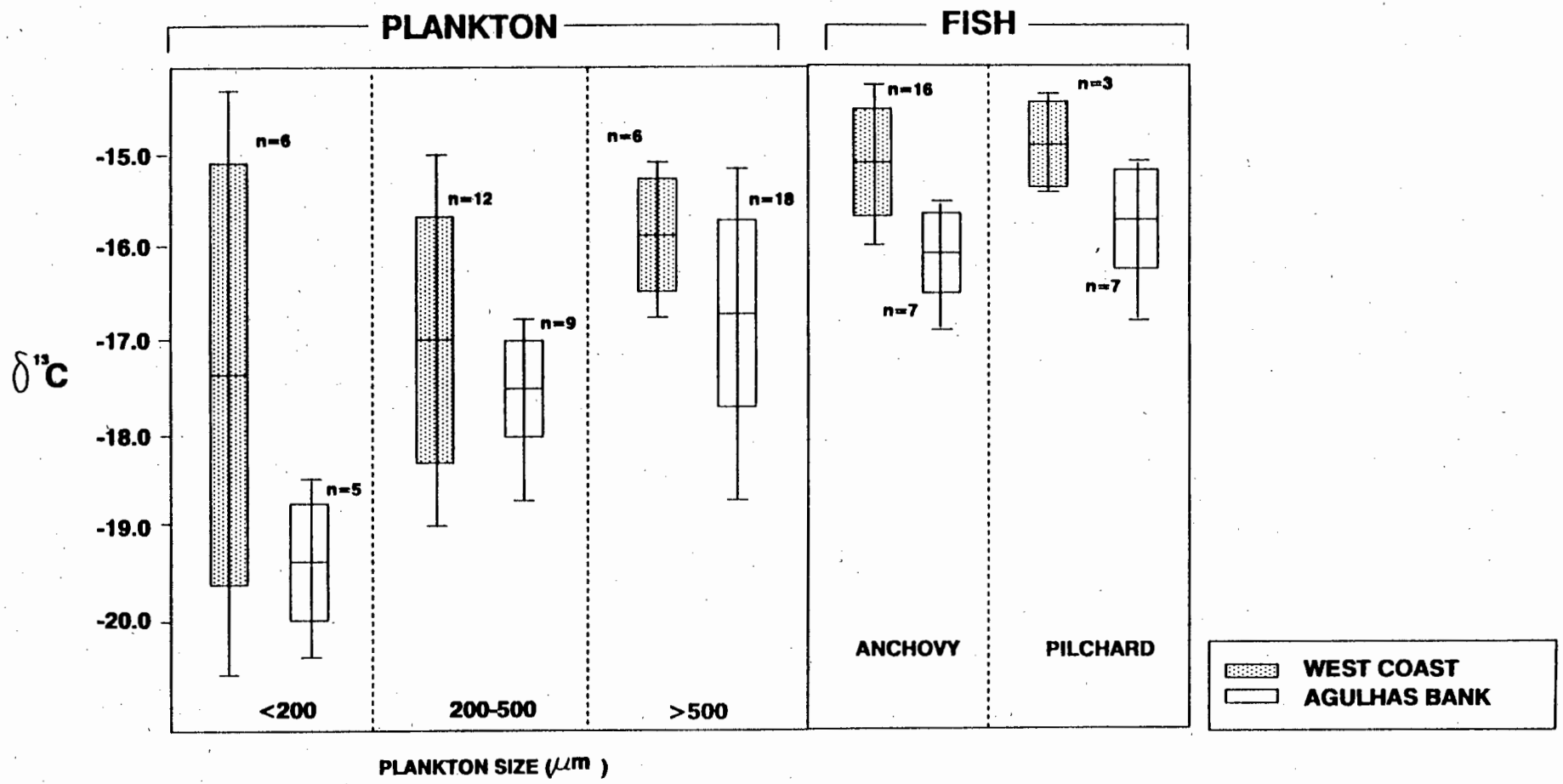


Fig. 26 $\delta^{13}C$ measurements for different size-classes of plankton and for the muscle tissue of Cape anchovy Engraulis capensis and sardine Sardinops ocellatus, from the Agulhas Bank area. Values for plankton from the west coast are included for comparison. All size-classes of fish are represented for muscle tissue. Vertical lines represent data ranges, rectangles represent standard deviations and the means are given by horizontal lines inside rectangles. Numbers of measurements (n) are also given.

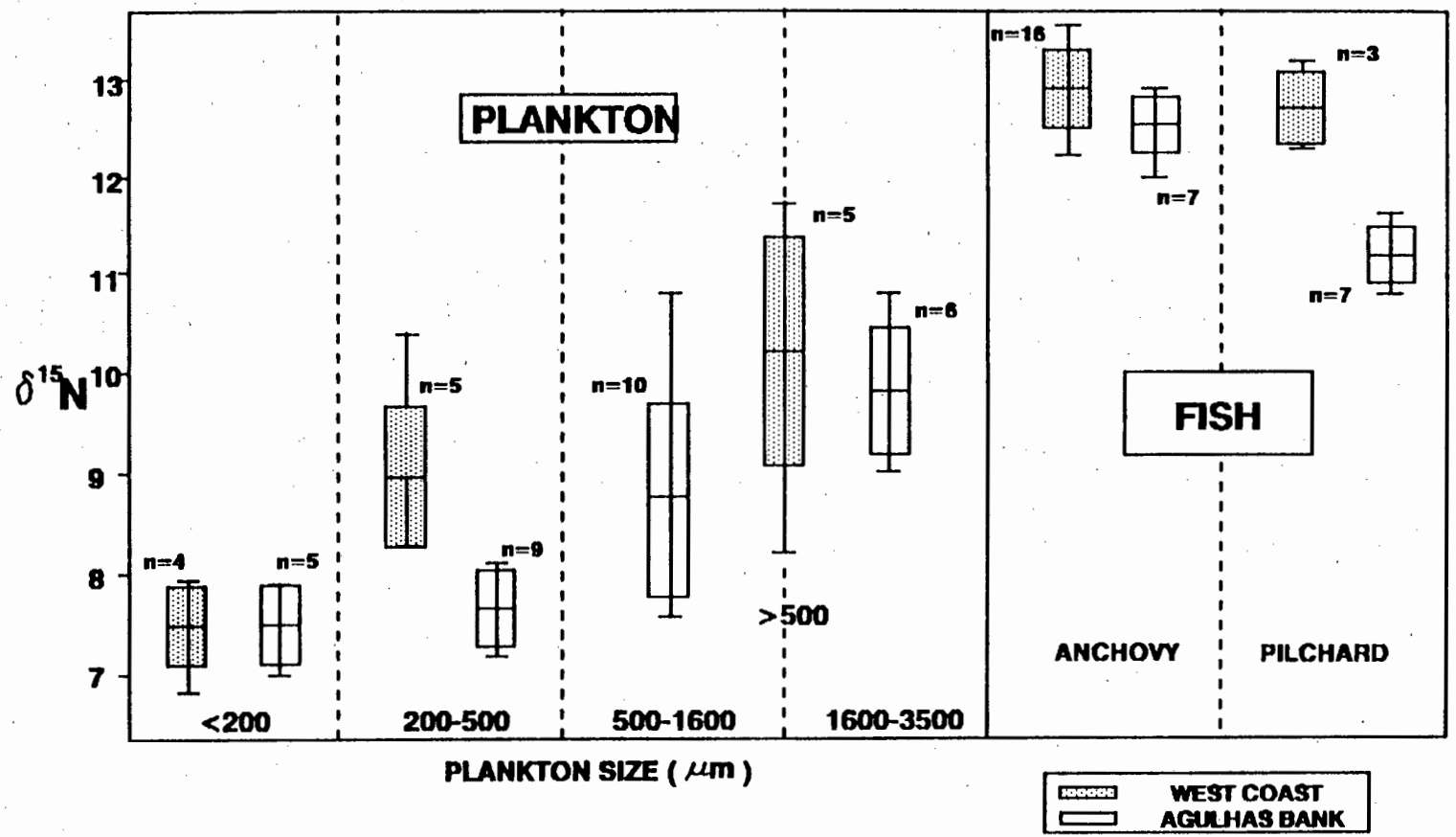


Fig. 27 $\delta^{15}N$ measurements for different size-classes of plankton and for the muscle tissue of Cape anchovy Engraulis capensis and sardine Sardinops ocellatus, from the Agulhas Bank area. Values for plankton from the west coast are included for comparison. All size-classes of fish are represented for muscle tissue. Vertical lines represent data ranges, rectangles represent standard deviations and the means are given by horizontal lines inside rectangles. Numbers of measurements (n) are also given.

Table 17 $\delta^{13}C$ statistics for the tissues of anchovy and sardine from the Agulhas Bank versus all size-classes of plankton from the same area, showing significant differences from Mann-Whitney-U tests (U = the test statistic for 1-tailed test, p = probability value for 1-tailed test) and Kruskal-Wallis one-way ANOVA (H = the test statistic, p = the associated probability value), ns = not significant.

ANCHOVY	vs	PLANKTON	SIGNIFICANCE	
Muscle	vs	$<200\ \mu m$	$U = 45,\ p<0.001$	$H=30.0, p<0.00005$
	vs	200-500 μm	$U = 70,\ p<0.0005$	
	vs	500-3500 μm	$U = 109,\ p<0.025$	
SARDINE	vs	PLANKTON	SIGNIFICANCE	
Muscle	vs	$<200\ \mu m$	$U = 35,\ p<0.0025$	$H=30.0, p<0.00005$
	vs	200-500 μm	$U = 62.5, p<0.0005$	
	vs	500-3500 μm	$U = 99.5, p<0.025$	

Table 18 $\delta^{15}N$ statistics for the tissues of anchovy and sardine from the Agulhas Bank versus all size-classes of plankton from the same area, showing significant differences from Mann-Whitney-U tests (U = the test statistic for 1-tailed test, p = significant for 1-tailed test) and Kruskal-Wallis one-way ANOVA (H = the test statistic, p = the associated probability value), ns = not significant.

ANCHOVY	vs	PLANKTON	SIGNIFICANCE	
Muscle	vs	<200 μm	U = 40, p<0.001	H=36.7, p<0.000001
	vs	200-500 μm	U = 72, p<0.0005	
	vs	500-1600 μm	U = 80, p<0.0005	
	vs	1600-3500 μm	U = 48, p<0.0005	

SARDINE	vs	PLANKTON	SIGNIFICANCE	
Muscle	vs	<200 μm	U = 35, p<0.0025	H=36.7, p<0.000001
	vs	200-500 μm	U = 63, p<0.0005	
	vs	500-1600 μm	U = 70, p<0.0005	
	vs	1600-3500 μm	U = 41.5, p<0.0025	

$\delta^{15}N$ values than the muscle tissue of anchovy from the same area ($U = 21$, $p<0.01$), which was not the case for fish from the west coast.

As for the west coast data, the fish tissues have more positive $\delta^{13}C$ and $\delta^{15}N$ values than all size-classes of plankton. The $\delta^{13}C$ values for sardine and anchovy muscle tissue are significantly more positive than all size-classes of plankton (Table 17). The $\delta^{15}N$ values for sardine and anchovy muscle tissue are also more positive than those for plankton of any size (Table 18).

For anchovy and sardine the $\delta^{13}C$ disparity between muscle tissue and >500 μm plankton is of the order 0.6 to 0.9 $^{o}/_{oo}$, close to the diet-consumer spacing reported in the literature for $\delta^{13}C$. The difference is greater for the two smaller plankton size-classes (1.6 to 3.5 $^{o}/_{oo}$). Assuming a diet-consumer difference of ca. 1-2 $^{o}/_{oo}$ for $\delta^{13}C$, anchovy and sardine may have consumed 200-500 μm and >500 μm plankton. The $\delta^{15}N$ difference between the fish muscle and the different plankton size-classes is of the order of 3.1 to 5.5 $^{o}/_{oo}$ for all the size-classes less than 1600 μm. Allowing for a 3-5 $^{o}/_{oo}$ disparity for $\delta^{15}N$, both fish species may have consumed plankton in any size-class less than 1600 μm. The difference between 1600-3500 μm plankton and fish muscle is 2.7 $^{o}/_{oo}$ for anchovy and 1.4 $^{o}/_{oo}$ for sardine. Hence anchovy probably consumed more 1600-3500 μm plankton than sardine.

SUMMARY

Larger plankton feed further up the foodweb than smaller plankton. The scatter in the data reflects the large number of trophic interactions within the pelagic foodweb.

The fish tissues have more positive $\delta^{13}C$ and $\delta^{15}N$ values than their gut contents and the disparity between the gut contents and fish tissues is similar for anchovy and roundherring. If one takes into account the life history of the fish, there is a general isotopic similarity between the tissues of the different species of fish suggesting that their isotopic make-up is characteristic of their position in the foodweb. Short term dietary differences due the fish's age, position, feeding periodicity, etc. may cause the observed differences in the $\delta^{13}C$ or $\delta^{15}N$ values of the gut contents of the different species. It appears that anchovy were consuming a large proportion of 200-500 μm plankton, supporting James (1987) who found that zooplankton was considerably more important in the diet of E. capensis than phytoplankton. The isotopic make-up of anchovy gut contents and tissues could, however, be the result of a combination of large (>500 μm) and small (<200 μm) plankton. Thus the results may not necessarily conflict with James' (1987) suggestion that E. capensis fulfills its nutritional requirements largely by selecting "mesozooplankton" between 1.0 and 20.0 mm (i.e. in the >500 μm size-class). It is unlikely however, that fish would simultaneously consume small and large plankton most of the time. There is no evidence that anchovy become predominantly phytophagous as has been suggested by King & Macleod (1976). Furthermore, the isotopic data support Cushing (1978) who suggested that King & Macleod (1978) overestimated the importance of phytoplankton in the diet of sardine.

Plankton and fish from the Agulhas Bank tend to have more negative values than those from the west coast. In the case of anchovy however, this is due to the size of the fish caught from each area. The ultimate cause may be dietary, but a physiological reason cannot be excluded.

CHAPTER 6

APPLICATION OF THE TROPHIC POSITION ISOTOPE SPECTRUM (T.P.I.S.)

INTRODUCTION

The Trophic Position Isotope Spectrum (T.P.I.S.) was conceived by Monteiro et al. (1991) from the $\delta^{13}C$ data reported in this study for the plankton and anchovy muscle samples collected from the southern Benguela ecosystem. The model helps to estimate, on the basis of isotopic data, the proportions of different size-classes of organisms in an organism's diet. I shall briefly describe the basic principles of the model and then illustrate its value. The approach attempts to define and quantify the pathways through which carbon, fixed by primary producers, flows to successive trophic levels. It makes use of frequency data rather than measures of central tendency. It hypothesises that the number of pathways along which carbon or nitrogen reaches a given compartment in the food-chain will be reflected by the distribution and frequency of occurrence of isotope ratios characterising organisms in that particular compartment. The isotope ratios cluster around δ values which are representative of the dominant input pathways during the period over which the organism's tissues integrate their dietary history. Since each data point is representative of the dietary history of the test organism, the technique is able to distinguish between individuals with different dietary histories (or from different populations).

In pelagic ecosystems there are different routes whereby primary consumer fish species are able to obtain phytoplankton carbon. They may feed directly on phytoplankton or consume planktivores further up the food web. Each pathway has an isotope ratio characteristic of the trophic position of the consumer organism and depends on the number of trophic steps (and therefore isotopic enrichment) from phytoplankton to fish. This is demonstrated hypothetically in Fig. 28 (A). The vertical axis represents increasing frequency of occurrence; the horizontal axis represents increasing trophic position. The relationship between ^{13}C enrichment and trophic position means that the pathway with the most steps has the most positive $\delta^{13}C$ values i.e. P3 > P2 > P1.

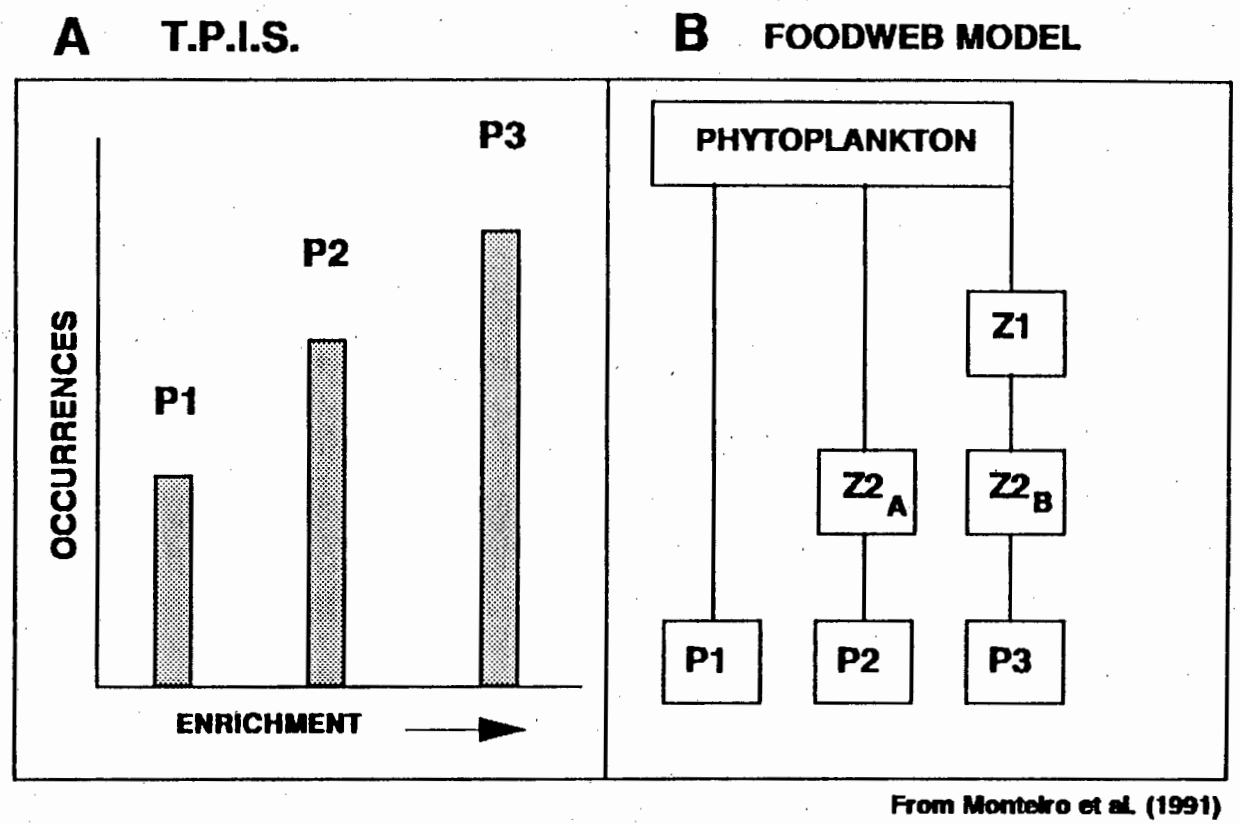


Fig. 28 Conceptual diagram of the relationship between (A) the trophic position isotope spectrum of a predator (P) and (B) its dietary relationship with the food chain (from Monteiro et al. 1991). It shows that for each pathway, there is a characteristic isotope ratio (P1, P2, P3) which is a function of the number of intermediary trophic steps and whose height indicates the number of individuals that have fed via that pathway. It is possible that an intermediate peak (P2) could also represent a mix of 2 extremes (P1 and P3). PHYTOPL represents phytoplankton; Z1 and Z2 are zooplankton trophic categories in this hypothetical foodweb. Z1 and $Z2_B$ are grazer categories and $Z2_A$ is a predator category.

6.1 $\delta^{13}C$ - Plankton to anchovy (southern Benguela region)

Fig. 29 shows the $\delta^{13}C$ T.P.I.S. for different size classes of plankton and anchovy tissues from the west coast (southern Benguela). The isotope ratios were rounded to within 0.125 $^{o}/_{oo}$ of each 0.25 $^{o}/_{oo}$ distribution interval. This was the maximum estimated experimental error and improved the signal-to-noise ratio without reducing the significance of natural differences (Monteiro et al. 1991). Within the plankton, the 20-200 μm size-class appears to show 3 trophic positions, identified by Monteiro et al. (1991) as "phytoplankton" at -19.75 to -20.50 $^{o}/_{oo}$ (the first trophic position), "microzooplankton" at -18.5 (μZA) and -15.5 to -15.75 $^{o}/_{oo}$ (μZB) (with more positive $\delta^{13}C$ values in the second and third trophic positions respectively). The dietary input for organisms in the μZA position appears to be from "phytoplankton" along a 1-step pathway (diet-consumer enrichment ca. 3 $^{o}/_{oo}$), while the diet for organisms in the μZB position appears to consist of organisms responsible for the μZA peak (a 2-step pathway with a diet-consumer difference of 3.5 $^{o}/_{oo}$).

The TPIS for the 200-500 μm size-fraction has two main peaks at -18.00 $^{o}/_{oo}$ (Z1) and -15.00 $^{o}/_{oo}$ (Z2) corresponding with the second and third trophic positions identified for μZA and μZB respectively. Similarly the dietary input for the Z1 and Z2 positions are likely to be from the "phytoplankton" and μZA positions respectively.

The frequency data for the >500 μm size-class (mZ2) cluster around -15.50 $^{o}/_{oo}$. The principle input pathways therefore appear to originate from organisms in the second trophic position (either μZA or Z1, $\delta^{13}C$ values of ca. -18 $^{o}/_{oo}$). The less positive $\delta^{13}C$ values in the TPIS of this plankton size-class (ca. -16.50) suggest that a less positive food source such as "phytoplankton", may have contributed to the carbon input of this size-class.

Monteiro et al. deduced from these data that organisms within the 20-200 and 200-500 μm size-classes consume phytoplankton along a 1-step pathway and zooplankton along a 2-step pathway. This accounts for the large scatter and standard deviations encountered amongst the data (see chapter 5). Phytoplankton appear less important as a dietary source for >500 μm plankton, since there is no mZ_1 component in this size-class. The diet-

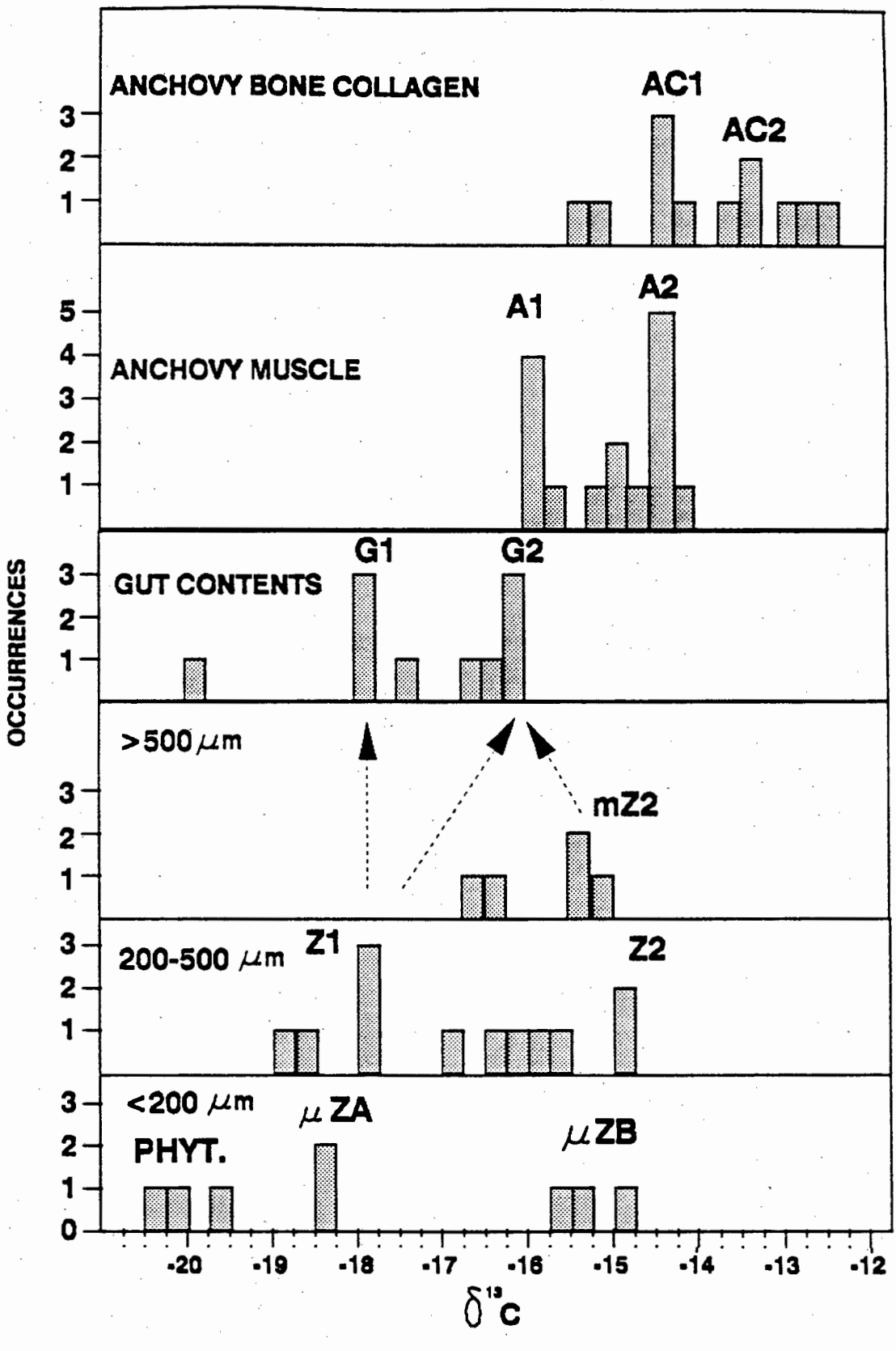


Fig. 29 Composite $\delta^{13}C$ TPIS diagram which includes anchovy (Engraulis capensis) gut contents and tissues and 3 size-classes of plankton samples (adapted from Monteiro et al. 1991). Organisms were caught from the west coast of South Africa. G1 and G2 designate the main peaks in the anchovy gut content TPIS, while A1 and A2, AC1 and AC2 designate the main peaks for anchovy muscle and bone collagen tissues respectively. Abbreviations for plankton are as in Fig. 28. Arrows indicate trophic pathways.

consumer differences between the plankton peaks are larger than those reported for whole organisms in the literature, which are usually < 2 $^{o}/_{oo}$. It is possible that these are not real diet-consumer relationships. On the other hand, frequency of occurrence data may provide a clearer picture of trophic interactions amongst plankton than do measures of central tendency by the ability to separate the different trophic categories in each size-class.

Strong bimodality is evident in $\delta^{13}C$ data for both the anchovy gut contents and tissues. The gut content data show peaks at -18.00 $^{o}/_{oo}$ and -16.25 $^{o}/_{oo}$ (a difference of 1.75 $^{o}/_{oo}$). The $\delta^{13}C$ peaks for anchovy gut contents, G_1 and G_2, appear to reflect a diet of organisms in the second trophic position (Z1 or μZ_A) and in the third trophic position (μZ_B, Z_2 or mZ_2) respectively. The position of the G_2 peak is slightly more negative than the plankton in the second trophic position, indicating that some less ^{13}C enriched organisms were present in the guts of the fish. The most negative value at -20.0 $^{o}/_{oo}$ suggests that "phytoplankton" may be consumed by the anchovy occasionally. The muscle tissue data cluster around -16.00 and -14.50 $^{o}/_{oo}$ (a 1.5 $^{o}/_{oo}$ difference). The isotopic spacing between the more negative $\delta^{13}C$ peaks for gut contents and muscle tissue is similar to that between the two more positive peaks (2 $^{o}/_{oo}$ and 1.75 $^{o}/_{oo}$ respectively). Anchovy bone collagen shows peaks at -14.5 and -13.5 $^{o}/_{oo}$, more positive than the corresponding muscle tissue peaks by 1.5 and 1 $^{o}/_{oo}$ respectively and more positive than the gut content peaks by 3.5 and 2.75 $^{o}/_{oo}$. The disparity between the anchovy tissues and gut contents are in keeping with the 2 $^{o}/_{oo}$ $\delta^{13}C$ enrichment factor from diet to consumer muscle, but slightly less than the 4.5 $^{o}/_{oo}$ ^{13}C enrichment from diet to consumer bone collagen tissue (Lee-Thorp et al. 1989).

Monteiro et al. concluded that anchovy obtain their food along a 2-step or 3-step pathway. In the case of the 2-step foodchain, they forage on the first trophic position (μZ_A, Z_1) and for the 3-step one, on second trophic position (μZ_B, Z_2, mZ). If we accept that the more positive μZA and μZB peaks in the 20-200 μm plankton size-class represent zooplankton species, then there is no evidence that the anchovy tissues reflect phytoplankton as a significant carbon source, supporting James (1987).

The $\delta^{13}C$ TPIS of Monteiro et al. (1991) illustrates how important information can be lost when using only measures of central tendency. Isotope values for the fish tissues may not

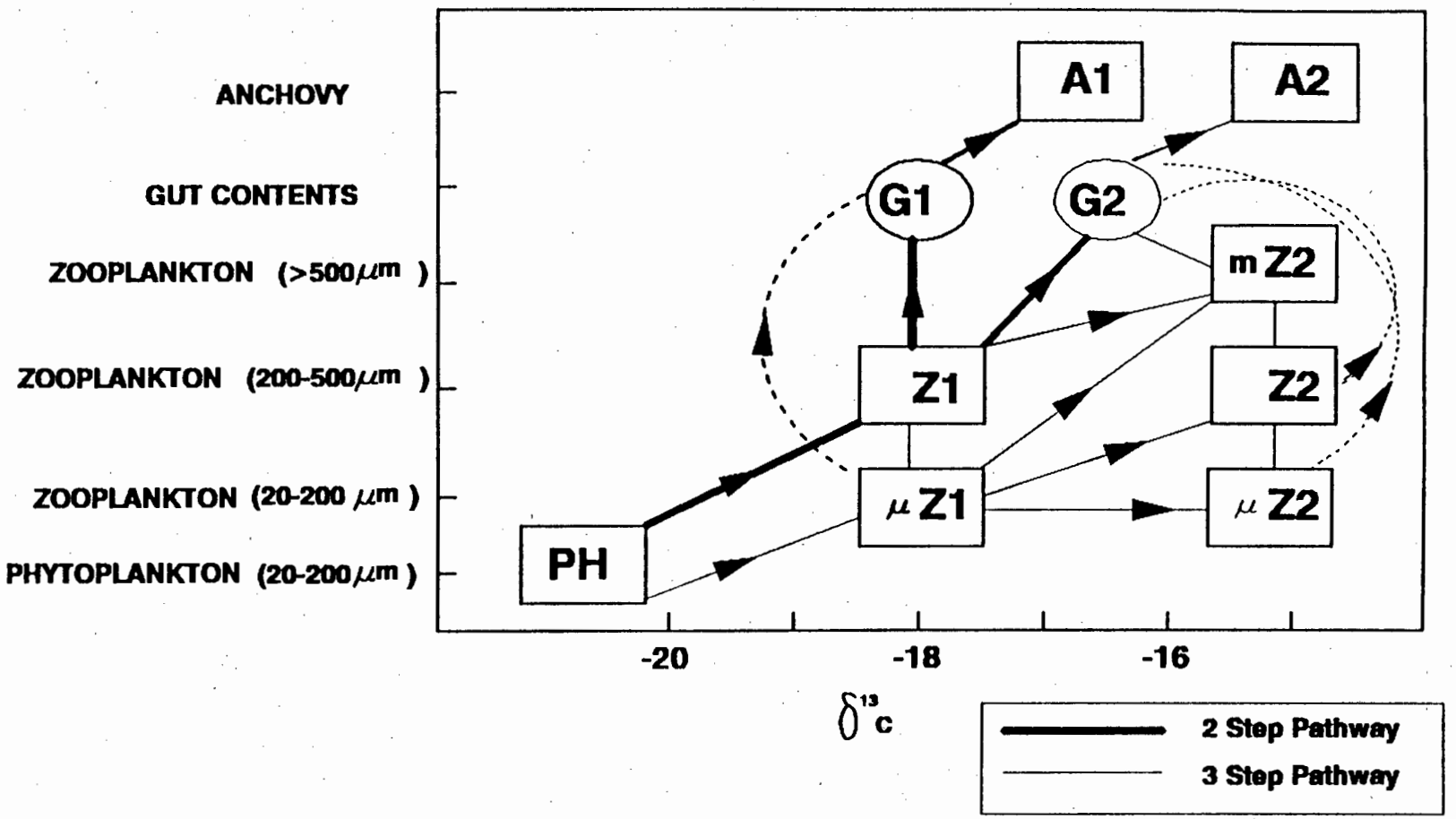


Fig. 30 Hypothesized isotope-based pelagic foodweb, constructed on the basis of the relationshpis of the compartments in the composite TPIS's for $\delta^{13}C$. Bold lines: 2-step pathways; thin lines: 3-step pathways; hatched lines: possible but unlikely pathways (from Monteiro et al. 1991). Abbreviations as in Fig. 29.

necessarily be more positive than those for plankton, as is indicated using the differences between mean values. Particular peaks within different size classes of plankton may correspond, despite the fact that the means for those size-classes may differ. Certain pathways are consistently important for each size-class of organisms. However, the frequency data between the peaks indicate dietary switching among the consumers. The data were compiled into a carbon flow network (Fig. 30). The dotted lines represent those pathways that have not been evidenced by gut content analyses.

Monteiro et al. (1991) point out that a problem with their model, is that the causes of the bimodality in the data need to be identified. Factors other than trophic enrichment may be responsible e.g. biogeography, fish size, season, etc. Measures of central tendency may be useful in situations where fish of different sizes show different amounts of fractionation relative to their gut contents (chapter 4). In the case of anchovy, the larger fish (particularly those >110 mm caudal length) are responsible for the more negative $\delta^{13}C$ peak (see Fig. 7). For gut contents however, the bimodality in the $\delta^{13}C$ TPIS is not related to fish length (Fig. 13 a). Thus the corresponding frequency distributions for gut contents and fish tissues may be co-incidental and the gut content values may not be a true reflection of the average diet consumed by the fish. Nevertheless it may be significant that anchovy show a preference for two isotopically distinct dietary components. If the sample size was large enough to plot a separate frequency distribution for the gut contents and tissues of each size-class of fish (as was the case for the plankton), the mismatch between the bimodal data distribution for the fish gut contents and fish tissues would be evident in the TPIS.

6.2 $\delta^{13}C$ - Plankton to roundherring (southern Benguela region)

The $\delta^{13}C$ TPIS for roundherring caught from the southern Benguela ecosystem can be seen in Fig. 31. There are fewer data for roundherring. Nevertheless, a $\delta^{13}C$ TPIS for roundherring muscle tissue shows a peak (R1) at -16.75 $^{o}/_{oo}$ (1 $^{o}/_{oo}$ more negative than the A1 peak for anchovy muscle tissue). The roundherring bone collagen values show a peak at -16.5, more positive than the muscle tissue peak (by only 0.25 $^{o}/_{oo}$), but more negative than the anchovy bone collagen peak, RC1, by 2 $^{o}/_{oo}$. More enriched data exists for both tissues, clustering in positions corresponding to the A2 and AC2 positions for

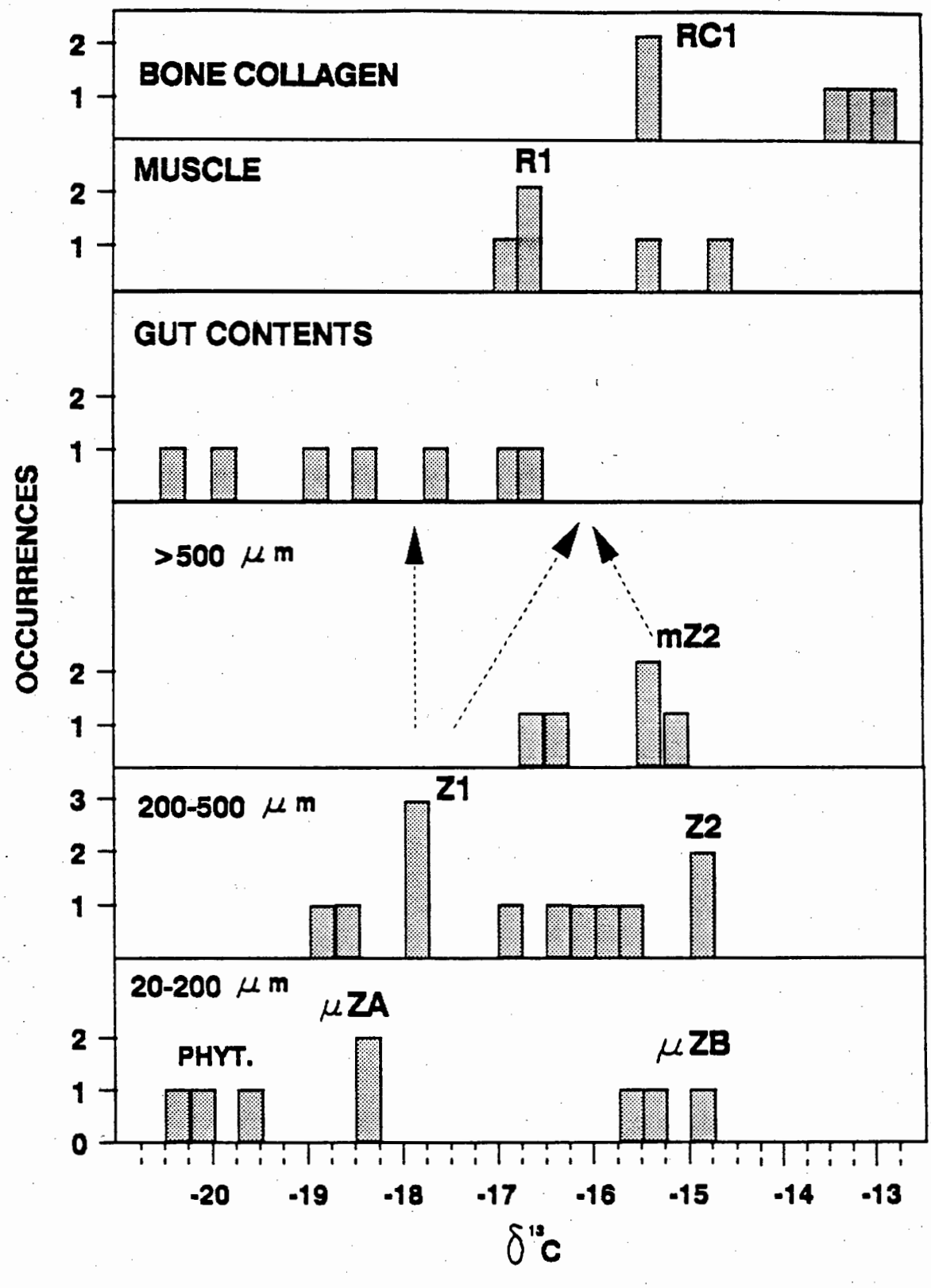


Fig. 31 Composite $\delta^{13}C$ TPIS diagram which includes roundherring (Etrumeus whiteheadi) gut contents and tissues and 3 size-classes of plankton. Organisms were caught from the west coast of South Africa. R1 and RC1 designate the major peaks for roundherring muscle and bone collagen tissues respectively. Other abbreviations as in Fig. 29.

anchovy. It is possible that given more data, the data distribution for roundherring tissues would be bimodal. It is surprising that this is evident with such few data (n = 5). As for anchovy, this is due to larger fish having more negative $\delta^{13}C$ values than smaller fish. As for anchovy, the isotopic relationship for gut contents with fish length does not correspond with that for the fish tissues. Roundherring gut contents show no clustering around any particular $\delta^{13}C$ value. These fish were consuming a varied diet when they were caught, but little plankton in the third trophic position, unless in combination with a more negative food source.

6.3 $\delta^{13}C$ - Plankton to anchovy and sardine (Agulhas Bank area)

Fig. 32 shows the $\delta^{13}C$ TPIS for anchovy and sardine muscle (on the same TPIS graph) and plankton caught from the Agulhas bank. The $\delta^{13}C$ data for the three sardine from the west coast are shown for comparison. The μZ and mZ2 peaks are lacking amongst the plankton data. The plankton peaks become more positive with increasing plankton size-class. The peaks in the <200 μm and 200-500 μm size-classes (-19.5 $^{o}/_{oo}$ and -17.75 $^{o}/_{oo}$ respectively) correspond respectively with the "phytoplankton" and the Z1 peaks for those organisms caught from the southern Benguela region. The $\delta^{13}C$ disparity between these peaks is 1.75 $^{o}/_{oo}$. A mZ1 peak emerges in the >500 μm size-class (-16.75 $^{o}/_{oo}$), more positive than the 200-500 μm peak by 1 $^{o}/_{oo}$, but more negative than the mZ2 peak identified for plankton from the southern Benguela by 1.25 $^{o}/_{oo}$. The division of the >500 μm plankton into 500-1600 μm and 1600-3500 μm size-classes produces a peak for the 500-1600 μm size-class in the same position as that for the total >500 μm sized-class, but the peak for the 1600-3500 μm fraction (-16.0 $^{o}/_{oo}$) is slightly more positive (by 0.75 $^{o}/_{oo}$).

The TPIS plots for plankton in the <200 μm and 200-500 μm size-classes show the same pattern. The frequency data in both size-classes span 2 $^{o}/_{oo}$ with the major peak occurring in the center. This suggests that diet-consumer interactions at each trophic position in the TPIS spectrum may be significant.

The peaks for anchovy and sardine muscle are similar (-15.75 and -16.0 $^{o}/_{o}$ respectively), and in a similar position to the A1 peak for anchovy from the west coast, -16.0 $^{o}/_{oo}$ (no bimodality is evident). The dietary input to fish appears to be along the same less positive

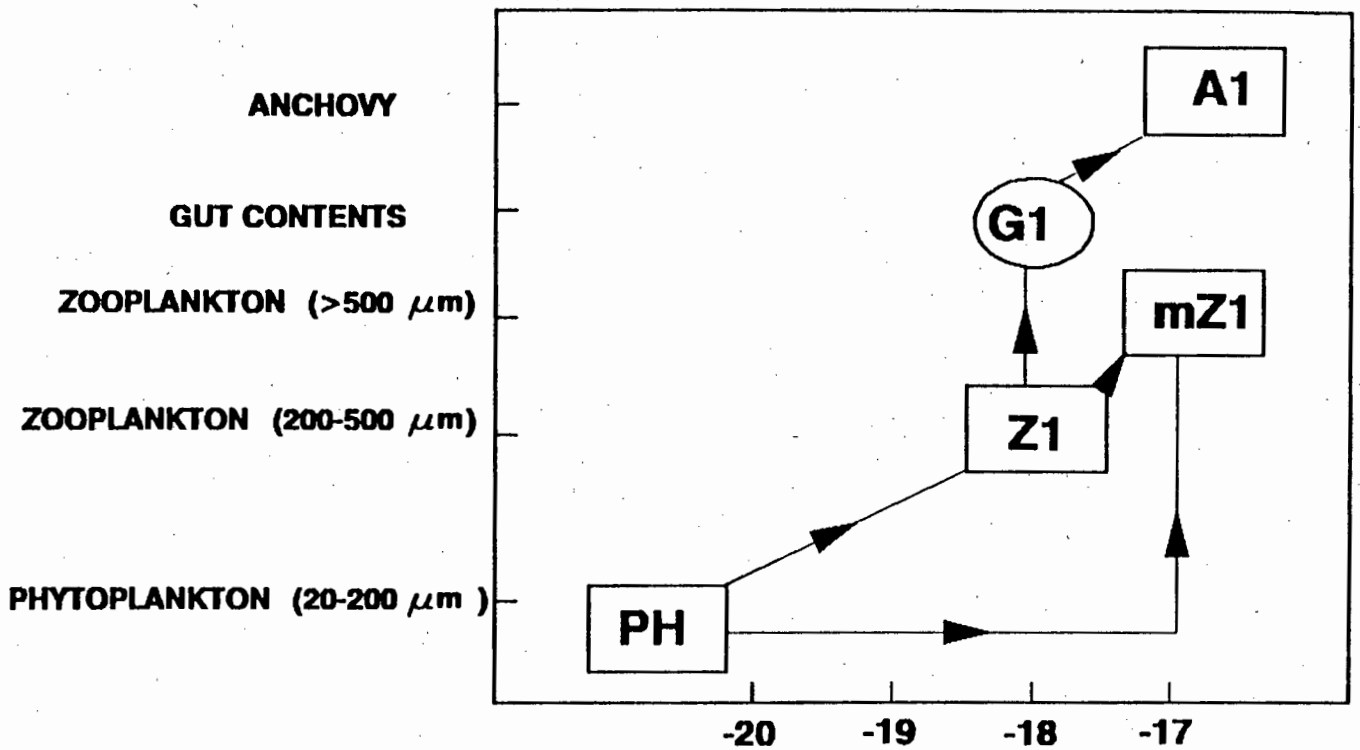


Fig. 33 Hypothesized isotope-based pelagic foodweb for the Agulhas Bank area constructed on the basis of the relationshpis of the compartments in the composite $\delta^{13}C$ TPIS's. Abbreviations as in Fig. 30.

two-step pathway as for organisms from the west coast. This results in the smaller standard deviations and more negative mean values for the Agulhas Bank data than was the case for organisms from the southern Benguela region. The lack of bimodality for the muscle tissue of anchovy from the Agulhas Bank is due to the size of fish caught in this area (anchovy from the Agulhas Bank are all greater than 110 mm caudal length). The fact that the distribution for plankton from this area is also bimodal suggests that the cause of the more negative values for larger fish is dietary. The larger fish, being closer to spawning age, are generally in a more southerly position, closer to the Agulhas bank and are more likely to reflect isotopically, a diet of plankton from this area than are smaller fish.

The $\delta^{13}C$ difference between the peaks for the different size-classes of plankton are less than the diet-consumer differences found between plankton peaks in the $\delta^{13}C$ TPIS for west coast organisms and closer to the 1-2 $^{o}/_{oo}$ reported in the literature. It is possible that there is more dietary overlap amongst the organisms from the Agulhas Bank. For instance the dietary input for organisms constituting the mZ1 peak may be partly a one-step (from <200 μm plankton) and partly two step pathway (from 200-500 μm organisms), resulting in a less ^{13}C enriched trophic position than the mZ2 which was present for organisms in this size-class from the west coast.

The combined data for the muscle tissue of sardine from the west coast and those from the Agulhas Bank results in the appearance of a bimodal frequency distribution for $\delta^{13}C$, with peaks corresponding in position to those for anchovy muscle. Sardine from the west coast are responsible for the more positive $\delta^{13}C$ enriched peak. The sizes of sardine from the two areas overlap, so it is not possible to conclude that the bimodality is size-related. Nevertheless the smaller fish from the Agulhas Bank were responsible for the more negative peaks, while the largest individual from the west coast was in the region of the more positive data.

The carbon flow model for the Agulhas Bank area can be seen in Fig. 33. The lack of the more positive $\delta^{13}C$ pathway results in a simpler foodweb with fewer trophic interactions. Species composition may ultimately be responsible for the differences between the two areas. The plankton from the Agulhas Bank consisted mainly of copepod species. The

lack of Euphausiids may be responsible for the lack of more ^{13}C enriched data for zooplankton.

6.4 $\delta^{13}C$ - Plankton to all fish species (both areas together)

Fig. 34 shows the $\delta^{13}C$ TPIS for all the fish and plankton data (both areas together). In each size-class of plankton there is a tendency to cluster around a mean frequency value. The plankton tend to have more positive $\delta^{13}C$ values with increasing size-class. The $\delta^{13}C$ differences between the major peaks for different size-classes of plankton are smaller than those found between the different trophic positions of plankton from each area separately. The difference between the major peak for <200 μm plankton (that corresponding to the μZA peak in Fig. 29) and that for 200-500 μm plankton is 0.5 $^{o}/_{oo}$ and the difference between the 200-500 μm and >500 μm (mZ1) size-classes is 1.25 $^{o}/_{oo}$, similar to the diet-consumer $\delta^{13}C$ disparity reported in the literature. Combining the data for all the organisms from both areas together reduces the relative importance of frequency distribution pathways for one species from each area.

Within the <200 μm plankton size-class, the data equivalent to that identified as "phytoplankton" from the west coast shows two peaks, one at -20.5 and one at -19.5 $^{o}/_{oo}$. The frequency data for the 200-500 μm size class show peaks around -18.75 $^{o}/_{oo}$ and -18 to -17.75 $^{o}/_{oo}$, corresponding with the peaks in the <200 μm plankton (allowing for ca. 1.25 $^{o}/_{oo}$ trophic enrichment).

The frequency distribution in the $\delta^{13}C$ TPIS for muscle tissue is biased towards the large number of anchovy samples relative to the other fish species. Measurements were not made for the gut contents and bone collagen tissue of fish from the Agulhas Bank. Furthermore the addition of the $\delta^{13}C$ values for anchovy from the Agulhas Bank results in a larger A1 peak for muscle tissue. The fish may have consumed slightly different average diets due to their position in the water column, age, etc., causing a less clear bimodal trend for the bone collagen data, which reflects a longer period of isotopic integration. The most interesting revelation is the emergence of three peaks (G, G1 and G2) within the $\delta^{13}C$ TPIS for gut contents. The gut contents of all three species of fish represent all the trophic positions evident in the plankton data. The two more positive peaks correspond with those for anchovy gut contents. Roundherring and sardine data

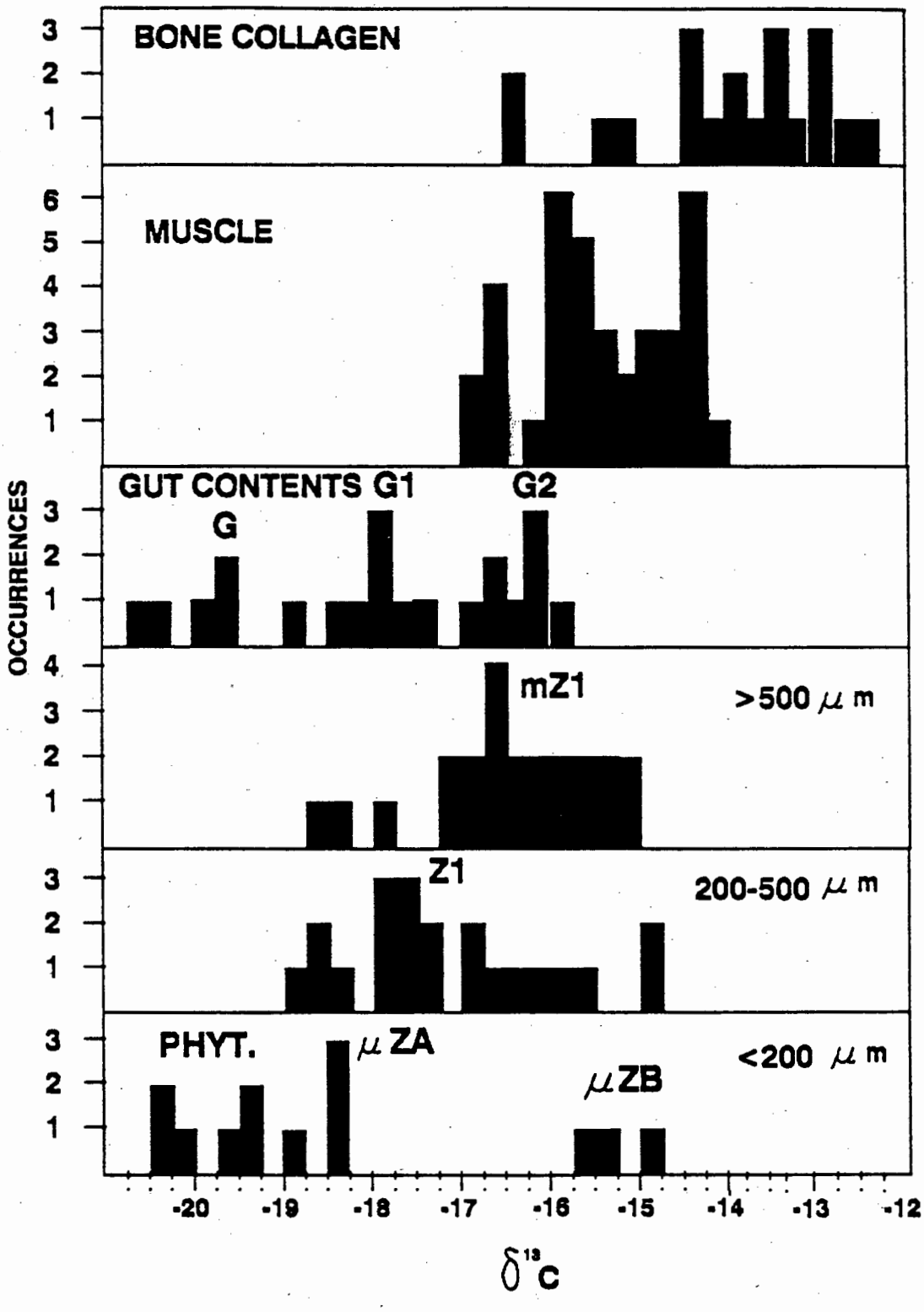


Fig. 34 Composite $\delta^{13}C$ TPIS diagram which includes anchovy (Engraulis capensis), roundherring (Etrumeus whiteheadi) and sardine (Sardinops ocellatus) tissues and 3 size-classes of plankton. Organisms from both the west coast and Agulhas Bank region are represented together (no roundherring were caught from the Agulhas Bank area). Abbreviations as for previous TPIS plots.

are responsible for the more negative peak at -19.75 $^{o}/_{oo}$. The $\delta^{13}C$ values for gut contents indicate that feeding directly on phytoplankton may be more important for roundherring and sardine than for anchovy.

TPIS plots for $\delta^{15}N$

6.5 $\delta^{15}N$ - Plankton to anchovy (southern Benguela region)

Fig. 35 shows the $\delta^{15}N$ TPIS for plankton and anchovy from the west coast. In the case of $\delta^{15}N$, the isotope ratios were rounded to within 0.25 $^{o}/_{oo}$ of each 0.5 $^{o}/_{oo}$ distribution interval because the measurement error (standard deviation) for similar sample material was found to be slightly larger for $\delta^{15}N$ than $\delta^{13}C$ (i.e. closer to 1.0 $^{o}/_{oo}$). One major peak is evident in the data for each plankton size-class. There is a 1 $^{o}/_{oo}$ increase from the peak for 20-200 μm plankton to that for 200-500 μm plankton (7.5 to 8.5 $^{o}/_{oo}$ respectively) and a 2 $^{o}/_{oo}$ increase from 200-500 μm to >500 μm plankton (at 10.5 $^{o}/_{oo}$). The major peaks in each plankton size-class are sequenced in such a way as to suggest trophic links from a "phytoplankton" group in the 20-200 μm size-class, to a Z1 type group in the 200-500 μm size-class, to a mZ2 group in the >500 μm size-fraction, but the disparities are smaller than diet-consumer relationships reported in the literature for $\delta^{15}N$ (at 3-5 $^{o}/_{oo}$), which contrasts to the situation for $\delta^{13}C$. A high degree of dietary overlap amongst planktonic organisms results in smaller diet-consumer enrichments between different species, depending on the proportion of phytoplankton in their diet (Kling & Fry 1992). There is no evidence of the μZ_1, μZ_2 in the <200 μm plankton size-class and therefore no Z_2 peak in the 200-500 μm size-class, which explains why the confidence intervals are smaller for $\delta^{15}N$ than $\delta^{13}C$ (chapter 5). There are fewer data points for $\delta^{15}N$ than $\delta^{13}C$ for plankton from the southern Benguela, since some samples were too small for $\delta^{15}N$ analysis using the equipment available at the time. The more positive $\delta^{13}C$ data responsible for the μZA and μZB positions in the $\delta^{13}C$ TPIS, may be due to the presence of mature phytoplankton with more positive $\delta^{13}C$ values due to reduced RUBISCO activity (chapter 1). If so, both the less and more positive $\delta^{13}C$ pathways represent a 2-step dietary links from phytoplankton to fish. It is interesting to note that those organisms responsible for the more positive $\delta^{13}C$ values are also those

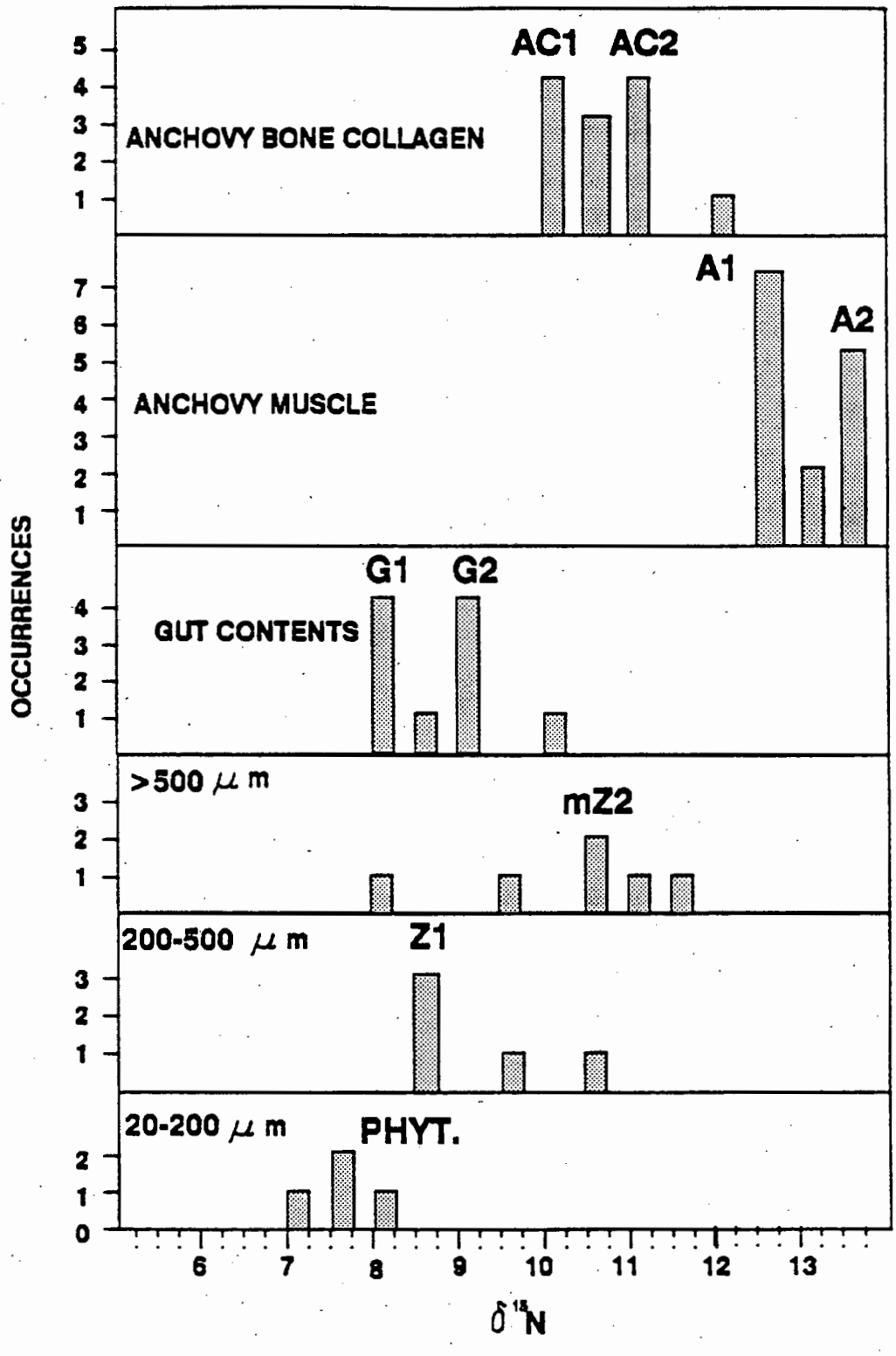


Fig. 35 $\delta^{15}N$ TPIS diagram including anchovy (Engraulis capensis) gut contents and tissues and 3 size-classes of plankton. Organisms were caught from the west coast of South Africa. Abbreviations as for Fig. 29.

responsible for the majority of the $\delta^{15}N$ data within both the 20-200 μm and 200-500 μm plankton size-classes (appendix 5).

There is a suggestion of a bimodal data distribution in the $\delta^{15}N$ TPIS for anchovy gut contents and tissues. The $\delta^{15}N$ increase from the gut content peaks to the corresponding muscle peaks is the same, at 4.5 $^{o}/_{oo}$. The peaks for bone collagen (at 10 $^{o}/_{oo}$ and 11 $^{o}/_{oo}$) are more positive than the gut content peaks by 2 $^{o}/_{oo}$ (more negative than the corresponding muscle peaks, at 12.5 $^{o}/_{oo}$ and 13.5 $^{o}/_{oo}$, by 2.5 $^{o}/_{oo}$ in each case). As for $\delta^{13}C$, it is the larger fish that are responsible for the more negative $\delta^{15}N$ peaks within the tissue data (chapter 4). The bimodality is least clear in the TPIS for bone collagen probably because dietary changes during the life of these fish (King & Macleod 1976) would be less isotopically clear for bone collagen than muscle tissue, due to its relatively slow turnover rate and longer period of isotopic integration.

The $\delta^{15}N$ TPIS for anchovy gut contents suggests a diet of mostly 200-500 μm plankton. However, the TPIS shows how a diet made up of a mixture of >500 μm and 20-200 μm plankton may produce the $\delta^{15}N$ TPIS for gut contents. For instance the G1 peak may consist of 20-200 μm and 200-500 μm plankton, while the G2 peak may be due to a combination of 200-500 μm plankton and >500 μm plankton. Smaller and larger anchovy may consume a mixture of plankton from different size-classes in such as way that, in the long term, larger fish obtain more negative isotope values. This could be due to the inclusion of increasing proportions of phytoplankton in the diet of larger anchovy, as is suggested by King & Macleod (1976). It is interesting that the pathways in the carbon flow model that have not been evidenced by gut content analyses (dotted lines), happen to be those that are absent from $\delta^{15}N$ TPIS (i.e. the two microzooplankton positions and the Z2 position).

6.6 $\delta^{15}N$ - Plankton to roundherring (southern Benguela region)

No bimodality is evident in the $\delta^{15}N$ TPIS plots for the gut contents and tissues of roundherring from the southern Benguela ecosystem (Fig. 36). As for anchovy, the position of the peak for roundherring gut contents suggests that they were consuming plankton mostly in the 200-500 μm size-class (in the second trophic position), but

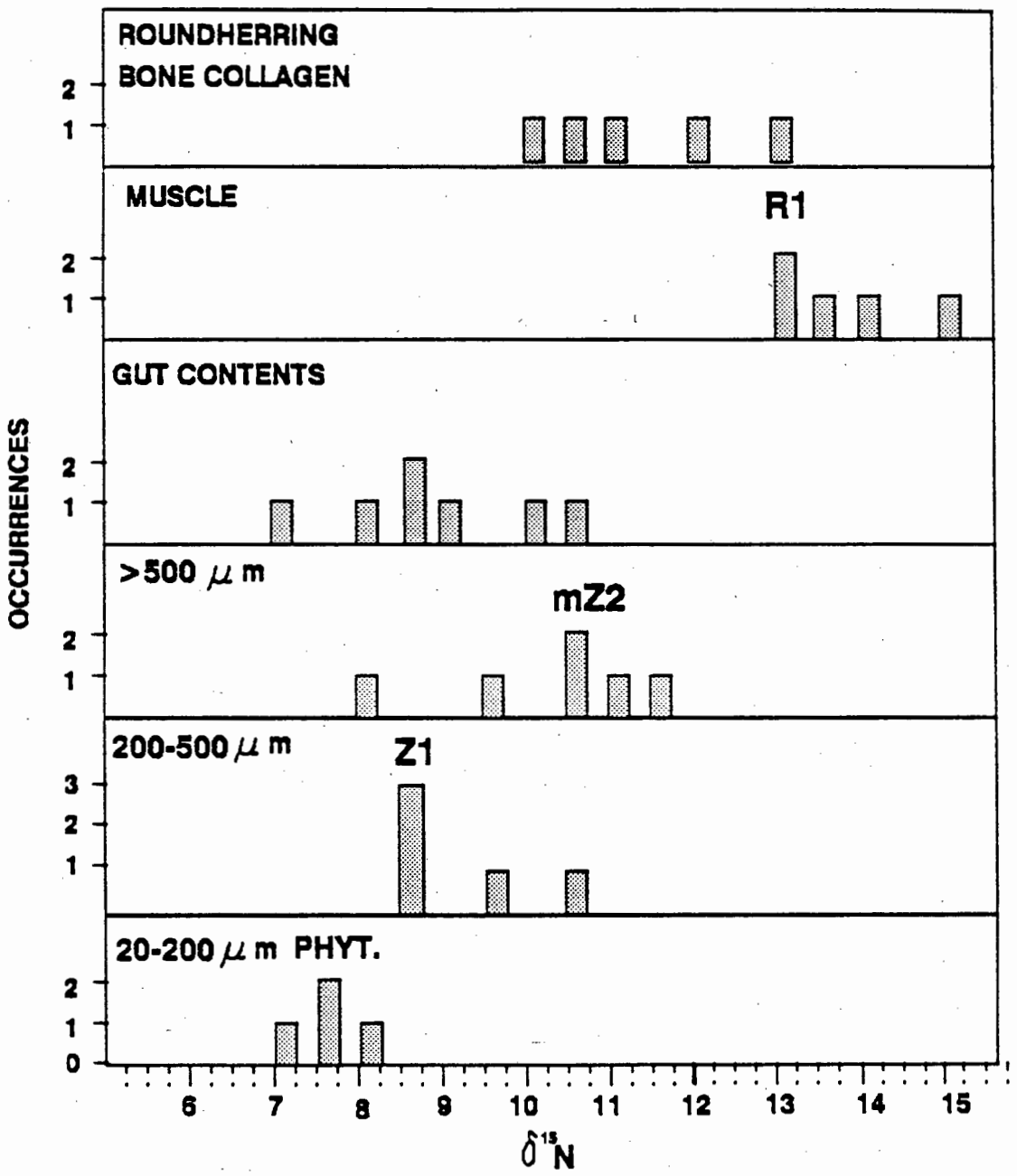


Fig. 36 Composite $\delta^{15}N$ TPIS diagram which includes roundherring (Etrumeus whiteheadi) gut contents and tissues and 3 size-classes of plankton. Organisms were caught from the west coast of South Africa. Abbreviations as in Fig. 31.

plankton from the 20-200 and >500 μm size-classes also contribute to their diet. The $\delta^{15}N$ data for muscle tissue show a peak at 13.0 $^{o}/_{oo}$, more positive than the gut content peak by 4.5 $^{o}/_{oo}$. The peaks for muscle tissue and gut contents fall between the corresponding $\delta^{15}N$ peaks for anchovy tissues (i.e. 0.5 $^{o}/_{oo}$ greater than the G1 and A1 peaks and 0.5 $^{o}/_{oo}$ smaller than the G2 and A2 peaks), indicating an average diet similar in $\delta^{15}N$ to that for anchovy. The bone collagen values are more negative than those for muscle tissue and show a similar range as for anchovy bone collagen, but no clustering around any particular trophic position.

6.7 $\delta^{15}N$ - Plankton to anchovy and sardine (Agulhas Bank area)

The $\delta^{15}N$ TPIS for anchovy and sardine muscle and plankton from the Agulhas Bank is shown in Fig. 37 No bimodality is evident in the $\delta^{15}N$ TPIS for the Agulhas Bank plankton, as is the case for their corresponding $\delta^{13}C$ values. Furthermore the increase in $\delta^{15}N$ with increasing plankton size class is less clear, except for the 1600-3500 μm size-class, which shows an increase by ca. 2 $^{o}/_{oo}$ (peak at 10.0 $^{o}/_{oo}$) relative to the smaller plankton size-classes. The major peaks in the <200, 200-500 and 500-1600 μm size-classes all correspond in position (ca 7.5-8.0 $^{o}/_{oo}$) suggesting that they consume similar diets and do not show much discrimination against ^{15}N. It is likely that a high degree of omnivory amongst the 200-500 μm and 500-1600 μm plankton exists (Kling & Fry 1992) and to a greater extent than for west coast species. Furthermore nutrient stress, or lack of available phytoplankton for organisms further up the foodweb, may cause them to show less discrimination against ^{15}N. The $\delta^{15}N$ difference between 1600-3500 μm plankton and the smaller plankton size-classes is similar to the difference between >500 μm and 200-500 μm plankton from the west coast (2 $^{o}/_{oo}$). Thus it is likely that the plankton in the >500 μm size-class from the west coast were larger than 1600 μm in size. If so, the $\delta^{15}N$ TPIS for both areas would be more similar. As for the west coast, the more enriched peak in the 1600-3500 μm size-class probably represents a two-step dietary pathway to these plankton (mZ2 trophic position).

The $\delta^{15}N$ peaks for anchovy muscle tissue (12.5 $^{o}/_{oo}$ and 13.0 $^{o}/_{oo}$) cluster around similar trophic positions to the A1 and A2 peaks in the $\delta^{15}N$ TPIS for west coast anchovy. The $\delta^{15}N$ peaks for sardine muscle tissue (11.0 $^{o}/_{oo}$ and 11.5 $^{o}/_{oo}$) are both 1.5

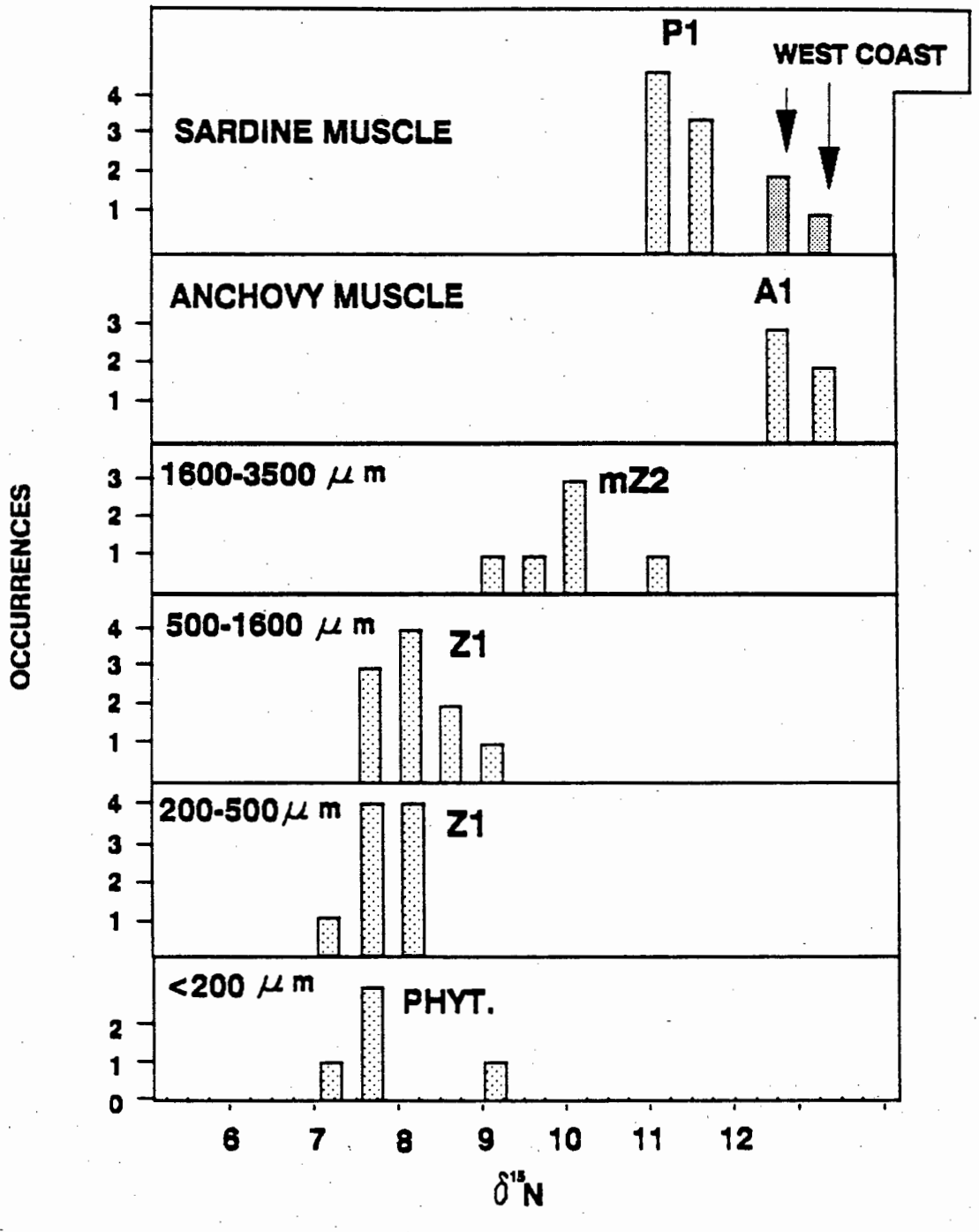


Fig. 37 Composite $\delta^{15}N$ TPIS diagram which includes anchovy (Engraulis capensis) and sardine (Sardinpos ocellatus) tissues and 3 size-classes of plankton. Organisms were caught from the Agulhas Bank area. Abbreviations as in Fig. 32.

$^{o}/_{oo}$ less than those for anchovy, suggesting that sardine consumed a diet more depleted in ^{15}N (but not ^{13}C). This may ultimately be due to the similarity in $\delta^{13}C$ between the 500-1600 μm and 1600-3500 μm plankton, while the $\delta^{15}N$ values for these size-classes of plankton differ. However, fractionation may differ for different species.

The $\delta^{15}N$ peaks for anchovy muscle tissue are more positive than those for plankton less than 1600 μm in size by ca. 4.5-5.5 $^{o}/_{oo}$ and more positive than 1600-3500 μm plankton by 2.5-3.0 $^{o}/_{oo}$. Sardine muscle tissue shows a slightly smaller $\delta^{15}N$ increase relative to the plankton, more positive than plankton less than 1600 μm in size by ca. 3.0-4.0 $^{o}/_{oo}$ and more positive than 1600-3500 μm plankton by 1 to 1.5 $^{o}/_{oo}$. Assuming a 4.5 $^{o}/_{oo}$ $\delta^{15}N$ disparity between diet (as gut contents at time of capture) and fish muscle, as was found for anchovy and roundherring from the west coast, then a large part of the diet of these fish consists of organisms less than 1600 μm in size. Plankton in the 1600-3500 μm size-class may also be consumed by the fish, particularly the more ^{15}N enriched anchovy.

6.8 $\delta^{15}N$ - Plankton to all fish species (both areas together)

Fig. 38 shows the $\delta^{15}N$ TPIS for all the fish and plankton data (both areas together). The $\delta^{15}N$ peaks for <1600 μm plankton correspond in position, while the peak for 1600-3500 μm plankton is more positive by ca. 2 $^{o}/_{oo}$ (if >500 μm plankton are represented as 1600-3500 μm plankton). The diet appears similar, on average, for the different species of fish. There is very little overlap between the $\delta^{15}N$ frequency data for muscle and bone collagen tissues. As for $\delta^{13}C$, increasing the number of larger anchovy, increases the height of the less enriched A1 peak. The sardine and roundherring data increase the height of those peaks situated between the peaks for anchovy muscle and those for anchovy bone collagen. There is a ca. 4.5-5 $^{o}/_{oo}$ increase from <1600 μm plankton to the main fish muscle peak. Assuming a 3-5 $^{o}/_{oo}$ $\delta^{15}N$ difference between the diet and consumer muscle tissue, the fish consumed plankton mostly <1600 μm in size. As for the $\delta^{13}C$ data, a more negative $\delta^{15}N$ peak (7 $^{o}/_{oo}$) emerges within the $\delta^{15}N$ TPIS for gut contents, confirming that roundherring and sardine are more likely to consume <200 μm plankton.

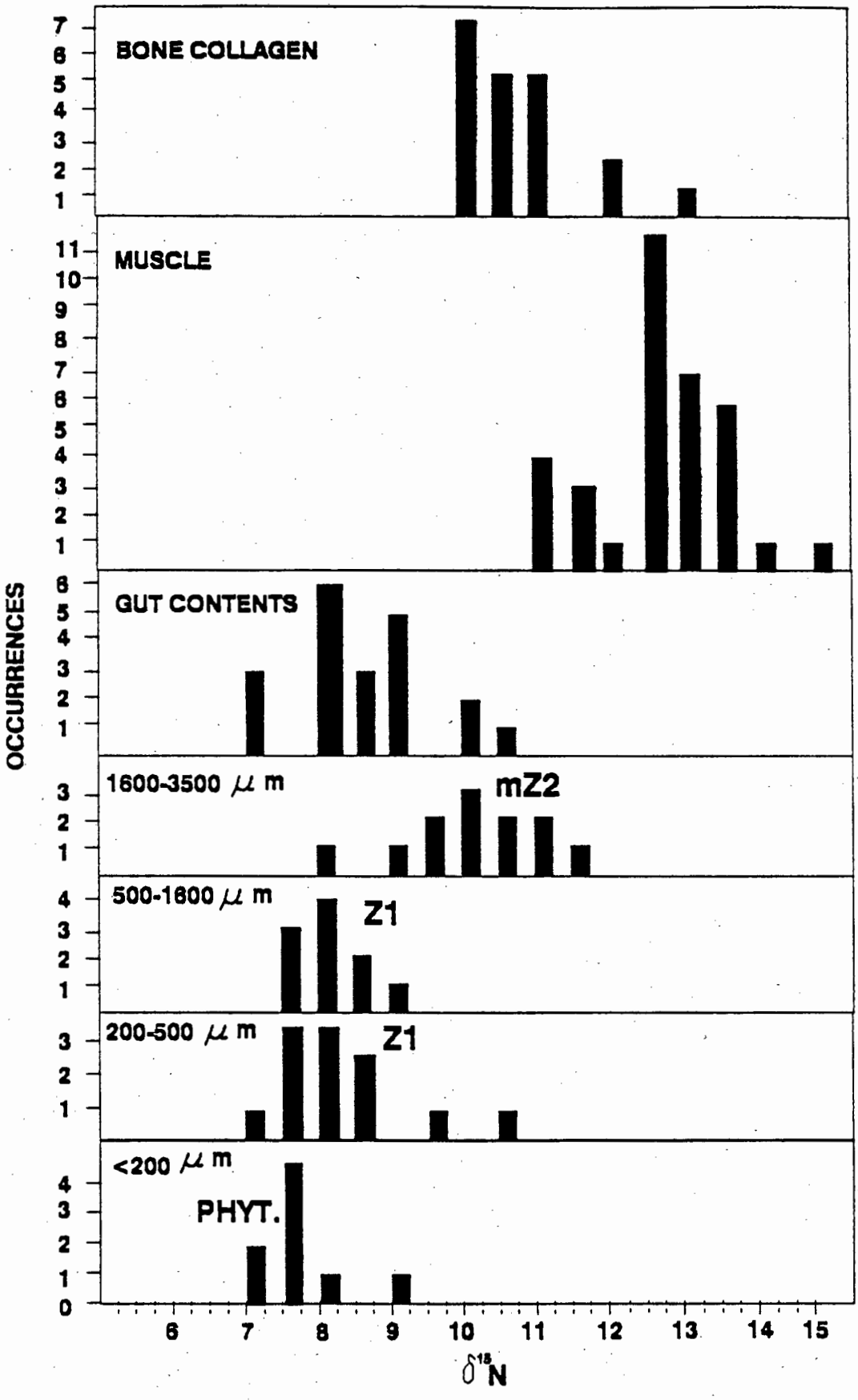


Fig. 38 Composite $\delta^{15}N$ TPIS diagram which includes anchovy (Engraulis capensis), roundherring (Etrumeus whiteheadi) and sardine (Sardinpos ocellatus) tissues and 3 size-classes of plankton. Organisms from both the west coast and Agulhas Bank region are represented together (no roundherring were caught from the Agulhas Bank area). Abbreviations as for previous TPIS plots.

SUMMARY

The TPIS developed by Monteiro et al. (1991) reveals important interactions within the pelagic foodweb. The $\delta^{13}C$ data reveal two dominant trophic pathways within the pelagic foodweb in the southern Benguela ecosystem, one with more positive $\delta^{13}C$ values in each size-class than the other. Only one of these pathways is evident among the $\delta^{15}N$ data, possibly due to the lack of data for $\delta^{15}N$. Only the more negative $\delta^{13}C$ pathway is evident among the data for organisms from the Agulhas Bank.

Larger anchovy prefer a diet isotopically similar to the more negative $\delta^{13}C$ pathway within the plankton. This diet is maintained when they move southwards to the spawning grounds in the vicinity of the Agulhas Bank, possibly due to species availability, since sardine from the Agulhas Bank also have more negative isotope values than those from the west coast. It is possible that larger fish consume greater proportions of phytoplankton than smaller fish. There is no suggestion that any of the fish species in this study are predominantly phytophagous.

The combination of the data for different species of fish masks interactions important for each individual species. Furthermore it is important to acquire a good sample size of different sized organisms for the TPIS to function adequately as a tool for interpreting dietary relationships amongst pelagic organisms.

The choice of biological tissue is important when interpreting TPIS data. Changes in the isotopic composition of an organisms diet will be reflected more quickly and precisely by muscle than bone collagen tissue, which will tend to blur the signals for a longer period of time.

CHAPTER 7

CONCLUSIONS

The data for plankton and pelagic schooling fish reported here re-affirm the usefulness of stable isotope ratios as indicators of trophic relationships in marine food webs. The data are consistent with the hypothesis that trophic relationships in pelagic food webs are governed largely by organism size (Cousins 1980, 1985; Azam et al. 1983; Platt 1985). Within the plankton community, there is trend whereby larger organisms have more positive $\delta^{13}C$ and $\delta^{15}N$ values, suggesting that they feed further up the foodweb than smaller organisms. Similarly, the muscle and bone collagen tissues of planktivorous fish species have more positive $\delta^{13}C$ and $\delta^{15}N$ values than plankton.

The presence of lipids in samples of plankton and anchovy, sardine and hake muscle cause them to have more negative $\delta^{13}C$ values. Planktonic organisms possess more lipid tissue than fish and show larger decreases in $\delta^{13}C$ (mean = 2.1 $^{o}/_{oo}$) than fish muscle (means = 0.8, 0.5 and 0.5 for anchovy, sardine and hake, respectively)). Of the fish, the $\delta^{13}C$ values of anchovy muscle are the most affected by lipids. Failure to remove lipids from samples may have serious effects on the interpretation of pelagic foodwebs, where diet-consumer relationships are usually ca. 1$^{o}/_{oo}$ for $\delta^{13}C$. In this study samples of different sizes were obtained from different seasons. All samples were defatted in order to reduce variability associated with differing lipid contents in these samples.

Experiments with adult sardine show that $\delta^{13}C$ and $\delta^{15}N$ turnover within the muscle and bone collagen tissues of pelagic fish is relatively slow, of the order of months or years, depending on the degree of isotopic disequilibrium with the food source. The turnover of nitrogen, and possibly carbon, in sardine muscle tissue, is faster than that in their bone collagen tissue. The average $\delta^{15}N$ turnover calculated was 0.6 $^{o}/_{oo}$ for sardine muscle tissue and 0.15 $^{o}/_{oo}$ for their bone collagen tissue. The turnover rate appears to slow down as isotopic equilibrium with their food source is approached. However a nutritionally inadequate food source may further retard the rate of turnover of carbon and nitrogen in the tissues of pelagic fish.

The average dietary histories of all three fish species are isotopically similar. The average preferred diets of pelagic fish species, as indicated by the isotope data for their tissues, do not necessarily coincide with that for their gut contents which are temporally biased. Stable isotope analyses provide a better estimate of the "preferred" diet of pelagic fish than conventional gut content analyses. None of the species are predominantly phytophagous. Anchovy appear to consume mostly 200-500 μm plankton, but larger anchovy may also consume phytoplankton and zooplankton greater than 500 μm in size. Roundherring and sardine appear to consume more phytoplankton, on average, than do anchovy.

The Trophic Position Isotope Spectrum allows identification of important interactions within the pelagic foodweb. Two main dietary pathways exist amongst the organisms from the southern Benguela ecosystem, one having more positive $\delta^{13}C$ values than the other. Only one pathway is evident amongst the organisms from the Agulhas Bank (the pathway with more negative $\delta^{13}C$ values). Different species compositions may be responsible for the presence of another pathway in the southern Benguela ecosystem but the time of year the samples were obtained may be important. Samples from the southern Benguela were obtained during autumn/winter, which signifies the end of the major upwelling and nutrient rich season. The associated decrease in nutrient availability would result in a decreased discrimination of the heavier isotopes (^{13}C and ^{15}N) by planktonic organisms. The complexity of the foodweb amongst the plankton from the southern Benguela ecosystem is reflected in the large standard deviations and the large standard errors for $\delta^{13}C$. According to the data in this study, the pelagic foodweb in the Agulhas Bank area appears simpler, with fewer trophic interactions.

The fractionation of $\delta^{13}C$ into the muscle and bone collagen tissues of the pelagic fish measured in this study differs to that for $\delta^{15}N$. Bone collagen acquires more ^{13}C than muscle and muscle tissue gains more ^{15}N than bone collagen. For anchovy and roundherring, the $\delta^{13}C$ difference between the potential diet and muscle tissue (2.1 $^{o}/_{oo}$ and 2.3 $^{o}/_{oo}$ respectively) is similar to the $\delta^{15}N$ difference between the potential diet and their bone collagen tissue (2.0 $^{o}/_{oo}$ and 2.5 $^{o}/_{oo}$ respectively). The ^{13}C enrichment to bone collagen is larger (3.2 $^{o}/_{oo}$ and 3.9 $^{o}/_{oo}$ for anchovy and roundherring respectively) and approaches that of the ^{15}N enrichment to muscle (4.3 $^{o}/_{oo}$ and 4.9 $^{o}/_{oo}$).

The muscle and bone collagen tissues of roundherring and anchovy have more negative $\delta^{13}C$ and $\delta^{15}N$ values with increasing fish size, suggesting that they consume more phytoplankton than smaller fish as is reported by King & Macleod (1976). Furthermore, their migratory position in relation to the available food sources may influence the dietary composition of different sized fish. The tissue with more negative $\delta^{13}C$ or $\delta^{15}N$ values (i.e. muscle or bone collagen respectively) shows the stronger negative correlation with increasing fish length. Hence one cannot exclude the possibility that the isotopic relationship with fish length has a physiological basis, the investigation of which is beyond the scope of this study.

Implications for future studies

The differences between the $\delta^{13}C$ and $\delta^{15}N$ content of muscle and bone collagen tissues and their relationships with fish size need further exploration. The physiological basis for inter- and intra-specific variability needs clarification.

Our ability to make quantitative inferences about diets will be substantially enhanced if we understand the mechanisms by which the isotopic make-up of biochemical components in the diet become incorporated into the biochemical components of consumers. The use of individual amino acids for stable isotope analyses has considerable advantages over analysis of bulk animal or plant tissue. The isotope ratios of separated amino acids generally have consistent relationships relative to one another from one animal to the next (Hare et al. 1991). A particular amino acid can be traced throughout the foodweb, reducing inaccuracies associated with different concentrations of amino acid types and the presence of lipids.

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APPENDIX 1 Turnover experiments

Sardinops ocellatus - Muscle

SAMPLE	DATE	δ 13C	δ 15N	
TROUT PELLET DIET:		-18.5	11.4	
1	1/11/89	-14.3	12.5	
2	8/11/89	-14.5	12.9	
3	13/12/89	-13.8	12.8	
4	11/1/90	-13.7	12.4	
5	31/1/90	-14.3	13.6	
	MEAN	-14.1	12.6	(n=5)
	S.D.	0.3	0.2	
ROE DIET:		-16.3	15.4	
1	25/3/90	-14.0	13.3	
2	31/3/90	-14.0	13.7	
3	8/4/90	-13.6	13.9	
4	16/5/90	-14.1	14.4	
5	5/6/90	-15.2	14.4	
6	3/7/90	-15.4	14.5	
	MEAN	-14.6	14.0	(n=6)
	S.D.	0.7	0.5	

Sardinops ocellatus - Bone collagen

SAMPLE	DATE	δ 13C	δ 15N	
TROUT PELLET DIET:		-18.5	11.4	
1	1/11/89			
2	8/11/89	-13.2	10.6	
3	13/12/89	-13.7	9.7	
4	11/1/90	-13.5	10.3	
5	31/1/90	-13.5	10.2	
	MEAN	-13.5	10.2	(n=4)
	S.D.	0.2	0.3	
ROE DIET:		-16.3	15.4	
1	25/3/90	-13.3	10.1	
2	31/3/90	-13.8	10.8	
3	8/4/90	-13.8	10.0	
4	16/5/90	-13.1	11.3	
5	5/6/90	-13.3	11.7	
6	3/7/90	-13.7	10.4	
	MEAN	-13.5	10.7	(n=6)
	S.D.	0.3	0.6	

APPENDIX 2

Data for anchovy (Engraulis capensis), from the west coast (southern Benguela)

sample number	fish length (mm)		$\delta^{13}C$	$\delta^{15}N$
GUT CONTENTS				
1	70-80	75.0	-16.4	10.0
2	70-80	75.0	-16.4	
3	70-80	75.0	-18.0	8.8
4	50-100	85.0	-16.2	8.9
5	90-100	95.0	-16.1	8.9
6	90-100	95.0	-18.1	8.2
7	100-120	110.0	-18.2	8.7
8	110-120	115.0	-19.9	7.9
9	110-120	115.0	-16.9	7.8
10	110-120	115.0	-17.5	7.8
11	100-150	125.0	-16.1	9.1
n=12		MEAN	-17.2	8.6
		S.D.	1.1	0.7
MUSCLE				
1	70-80	75.0	-14.6	12.6
2	70-80	75.0	-14.4	12.3
3	70-80	75.0	-14.9	12.5
4	70-80	75.0	-14.6	13.6
5	70-80	75.0	-14.6	13.1
6	90-100	95.0	-15.0	13.3
7	90-100	95.0	-14.8	13.5
8	100-110	105.0	-14.2	13.4
9	100-110	105.0	-15.3	13.3
10	100-120	110.0	-14.6	13.0
11	110-120	115.0	-15.7	12.4
12	110-120	115.0	-16.1	12.7
13	120-130	125.0	-16.0	12.7
14	120-130	125.0	-16.0	12.7
n=16		MEAN	-15.1	12.9
		S.D.	0.6	0.4
BONE:				
1	70-80	75.0	-12.7	11.0
2	70-80	75.0	-13.1	10.8
3	70-80	75.0	-14.4	10.3
4	70-80	75.0	-14.6	11.8
5	90-100	95.0	-13.4	10.5
6	100-110	105.0	-12.6	10.4
7	100-110	105.0	-13.7	10.9
8	100-110	105.0	-13.5	10.9
9	110-120	115.0	-14.6	9.8
10	110-120	115.0	-15.4	10.1
11	120-130	125.0	-14.1	10.2
12	120-130	125.0	-15.3	10.2
n=13		MEAN	-13.9	10.6
		S.D.	0.9	0.5

APPENDIX 3

Data for redeye roundherring (Etrumeus whiteheadi) from the west coast (southern Benguela)

SAMPLE	CAUDAL LENGTH (mm)	δ 13C	δ 15N
GUT CONTENTS			
1	110-120	-19.1	8.8
2	100-150	-17.2	8.4
3	140-150	-20.5	7.0
4	150-160	-20.1	8.5
5	150-160	-18.4	7.9
6	150-200	-16.7	10.6
7	150-200	-17.7	10.2
n=7	MEAN	-18.5	8.75
	S.D.	1.3	1.2
MUSCLE			
1	110-120	-14.9	15.1
2	110-120	-15.4	14.2
3	140-150	-17.1	12.9
4	150-160	-16.9	13.6
5	150-160	-16.8	12.8
n=5	MEAN	-16.2	13.7
	S.D.	0.9	0.8
BONE:			
1	110-120	-13.1	13.2
2	110-120	-13.6	11.8
3	140-150	-13.2	10.3
4	150-160	-16.6	11.2
5	150-160	-16.4	10.1
n=5	MEAN	-14.6	11.3
	S.D.	1.6	1.1

APPENDIX 4

Data for sardine (Sardinops ocellatus) from the west coast (southern Benguela)

SAMPLE	CAUDAL LENGTH (mm)	δ 13C	δ 15N
GUT CONTENTS:			
1	80-90	-22.7	7.2
2	160-170	-18.7	7.2
3	200-210	-19.7	7.8
n=3	MEAN	-20.4	7.4
	S.D.	1.7	0.3
MUSCLE			
1	80-90	-15.5	12.3
2	160-170	-14.5	12.6
3	200-210	-14.8	13.2
n=3	MEAN	-14.9	12.7
	S.D.	0.4	0.4
BONE:			
1	80-90	-14.3	10.2
2	160-170	-13.9	10.4
3	200-210	-13.1	10.1
n=3	MEAN	-13.8	10.2
	S.D.	0.5	0.1

APPENDIX 5

Data for plankton of different size-classes from the west coast (southern Benguela)

Size-class (microns)	δ 13 C	δ 15N	
20-200:	-14.2	7.6	
	-18.5	7.9	
	-15.5	7.5	
	-15.1	6.8	
	-20.6		
(n=6)	-19.8		(n=4)
MEAN	-17.3	7.5	
S.D.	2.4	0.4	
200-500:	-15.0	8.4	
	-15.1	8.5	
	-15.8	9.4	
	-16.0	10.5	
	-16.4	8.4	
	-16.3		
	-18.1		
	-18.8		
	-19.0		
	-17.9		
	-17.1		
(n=12)	-18.0		(n=5)
MEAN	-17.0	9.0	
S.D.	1.3	0.8	
>500:	-15.5	9.3	
	-15.6	10.6	
	-16.8	10.9	
	-15.7	10.4	
	-15.2	11.7	
(n=6)	-16.5		(n=5)
MEAN	-15.8	7.2	
S.D.	0.6	4.4	

APPENDIX 6

Data for different size-classes of plankton from the Agulhas Bank

SAMPLE	STATION	DATE	SIZE CLASS (microns)	δ13 C	δ 15N
1	31	28/11/89	<200	-19.1	7.6
2	29	28/11/89	<200	-18.5	7.0
3	27	27/11/89	<200	-19.5	7.5
4	31	28/11/89	<200	-19.4	7.7
5	3	23/11/89	<200	-20.5	9.2
			MEAN	-19.9 (n=5)	7.7 (n=5)
			S.D.	0.5	0.4
1	13	25/11/89	200-500	-18.4	7.9
2	14	26/11/89	200-500	-16.8	7.7
3	14	26/11/89	200-500	-16.9	8.1
4	28	27/11/89	200-500	-17.7	7.8
5	28	27/11/89	200-500	-17.7	7.6
6	28	27/11/89	200-500	-17.7	7.5
7	29	29/11/89	200-500	-17.4	8.0
8	31	28/11/89	200-500	-17.4	7.2
9	32	28/11/89	200-500	-18.8	7.5
			MEAN	-17.6 (n=9)	7.7 (n=9)
			S.D.	0.6	0.3
1	4	13/11/89	500-1600	-17.0	8.5
2	11	25/11/89	500-1600	-16.9	7.6
3	13	25/11/89	500-1600	-16.1	8.4
4	13	25/11/89	500-1600	-16.7	8.1
5	18	26/11/89	500-1600	-15.8	7.6
6	26	27/11/89	500-1600	-15.2	8.2
7	27	27/11/89	500-1600	-16.3	8.0
8	28	27/11/89	500-1600	-17.2	8.0
9	28	27/11/89	500-1600	-17.0	7.7
10	29	28/11/89	500-1600	-18.8	9.2
11	32	28/11/89	500-1600	-16.6	
			MEAN	-16.7 (n=11)	8.1 (n=10)
			S.D.	0.9	0.5
1	4	23/11/89	1600-3500	-15.7	10.0
2	13	25/11/89	1600-3500	-16.1	10.2
3	13	25/11/89	1600-3500	-16.0	10.8
4	13	25/11/89	1600-3500	-18.4	9.0
5	27	27/11/89	1600-3500	-16.4	10.0
6	27	27/11/89	1600-3500	-17.3	9.3
7	32	28/11/89	1600-3500	-18.0	
			MEAN	-16.8 (n=7)	9.9 (n=6)
			S.D.	1.0	0.6

APPENDIX 7

Data for sardine (Sardinops ocellatus) from the Agulhas Bank

MUSCLE TISSUE

SAMPLE	STATION	DATE	SIZE (mm)	δ 13C	δ 15N	
1	12-04	14/11/89	120-130	-16.2	11.0	
2	14-01	15/11/89	120-130	-16.1	11.6	
3	28-08	22/11/89	150-160	-16.8	10.8	
4	15-03	15/11/89	150-160	-15.5	10.9	
5	14-01	15/11/89	160-170	-16.0	11.2	
6	16-01	16/11/89	160-170	-15.3	11.3	
7	20-02A	19/11/89	200-210	-15.1	11.4	
			MEAN	-15.8	11.2	(n=7)
			S.D.	0.6	0.3	

GUT CONTENTS

1	12-04	14/11/89	120-130	-15.5	6.0	
2	28-08	22/11/89	150-160	-17.7	7.9	
3	28-08	22/11/89	150-160	-16.4	7.3	
			MEAN	-16.5	7.1	(n=3)
			S.D.	0.9	0.8	

HINDGUT CONTENTS

1	12-04	14/11/89	120-130	-15.5	8.7	
2	14-01	15/11/89	120-130	-15.4	9.2	
3	28-08	22/11/89	150-160	-17.3	9.1	
4	15-03	15/11/89	150-160	-15.5	9.0	
5	14-01	15/11/89	160-170	-15.4	9.9	
6	16-01	16/11/89	160-170	-15.1	8.7	
7	20-02A	19/11/89	200-210	-15.5	8.1	
			MEAN	-15.7	9.0	(n=7)
			S.D.	0.7	0.5	

APPENDIX 8

Data for anchovy muscle tissue (Engraulis capensis) from the Agulhas Bank

STATION	DATE	SIZE (mm)	δ 13C	δ 15N	
26-05A	20/11/89	110-120	-15.9	12.8	
26-05A	20/11/89	110-120	-15.9	12.1	
09-08A	13/11/89	110-120	-15.8	12.7	
40-05A	29/11/89	110-120	-16.9	12.0	
28-09A	22/11/89	110-120	-16.8	12.5	
28-09A	22/11/89	110-120	-15.6	12.9	
09-08A	13/11/89	120-130	-15.7	12.5	
		MEAN	-16.1	12.5	(n=7)
		S.D.	0.5	0.3	